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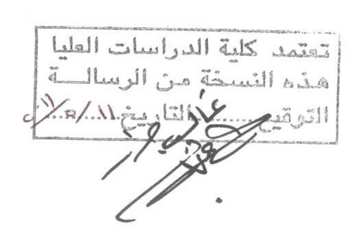
نموذج رقم (١٨)
اقرار والتزام بالمعايير الأخلاقية والأمانة العلمية
وقوانين الجامعة الأردنية وأنظمتها وتعليماتها
لطلبة الماجستير

أنا الطالب: جمانة أحمد نقلة لشوالكة الرقم الجامعي: (٨٠٨٠٩٢٢)
تخصص: علوم صيدلانية الكليـــــة: الصيدلة

عنوان الرسالة: Synthesis and optimization of new hormone sensitive lipase (HSL) Inhibitors as potential antidiabetic agents and hypolipidemic agent

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد رسائل الماجستير عندما قمت شخصيا" باعداد رسالتي وذلك بما ينسجم مع الأمانة العلمية وكافة المعايير الأخلاقية المتعارف عليها في كتابة الرسائل العلمية. كما أنني أعلن بأن رسالتي هذه غير منقولة أو مستلة من رسائل أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

توقيع الطالب: .... التاريخ: ١١ / ٥ / ٢٠١١



SYNTHESIS AND OPTIMIZATION OF NEW HORMONE SENSTIVE LIPASE (HSL) INHIBITORS AS POTENTIAL ANTIDIABETIC AGENTS AND HYPOLIPIDIMIC AGENTS.

By

Jumanah D. Al-Shawabkah

Supervisor

Dr. Mutasem O. Taha, Prof.

Co- Supervisor

Dr. Ghassan Abu Sheikha

This Thesis was submitted in Partial Fulfillment of the Requirements for the Master's Degree of Pharmacy in Pharmaceutical Sciences

Faculty of Graduate Studies

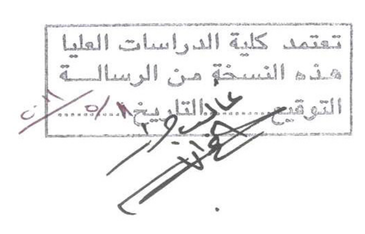
The University of Jordan

May, 2011

COMMITTEE DECISION

This Thesis (Synthesis and Optimization of New Hormone Sensitive Lipase (HSL) Inhibitors as potential antidiabetic agents and hypolipidimic agents) was Successfully Defended and Approved on 31/ 3/ 2011.

Examination Committee	**Signature**
Dr. Mutasem O. Taha (Supervisor) **Professor of Medicinal Chemistry and Drug Design**	------------------------
Dr. Ghassan Abu Sheikha (Co-supervisor) **Assist. Professor of Medicinal Chemistry**	------------------------
Dr. **Mohammad S. Mubarak (Member)** **Professor of Organic Chemistry**	------------------------
Dr. Ghadeer Suaifan (Member) **Assist. Professor of Medicinal Chemistry**	------------------------
Dr. Qosay A. Al-Balas, (Member) **Assist. Professor of Medicinal Chemistry,** **(Jordan University of Science and Technology)**	------------------------



Dedicated

To my beloved father & mother

For their continuous support & motivation

To my sisters, brothers, & friends;

Who helped & supported me whenever I needed them.

Acknowledgment

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List of Abbreviation

Number	Symbol or Abbreviation	Definition
1	HSL	Hormone Sensitive Lipase
2	FFAs	Free Fatty Acids
3	TAG	Triacylglycerol
4	PKA	Protein kinase A
5	cAMP	cyclic adenosine monophosphate
6	Ser	Serine
7	VLDL	Very low density lipoprotein
8	3D	Three dimensional
9	TEA	Triethaylamine
10	QSAR	Quantitative structure activity relationship
11	HBA	Hydrogen Bond Acceptor
12	HBD	Hydrogen Bond Donor
13	Ring Arom	Ring Aromatic
14	Hbic	Hydrophobic
15	Hypo	Hypotheses
16	GFA	Genetic Function Approximation
17	KRB	Krebs ringer bicarbonate
18	BSA	Bovine serum albumin
19	PNPB	*p*-nitrophenyl butyrate
20	DMAP	Dimethylaminopyridine
21	NMR	Nuclear Magnetic Resonance

SYNTHESIS AND OPTIMIZATION OF NEW HORMONE SENSTIVE LIPASE (HSL) INHIBITORS AS POTENTIAL ANTIDIABETIC AGENTS AND HYPOLIPIDIMIC AGENTS.

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ABSTRACT

Hormone sensitive lipase (HSL) has been recently implicated in diabetes and obesity prompting several attempts to discover and optimize new HSL inhibitors

The current effort includes preparation and biological testing of 49 compounds of aryl amide, aryl ester and sulfonamide derivatives guided by previously published anti-HSL pharmacophore models and QSAR equation. The prepraration procedures included coupling reactions involving phenols, anilines and aryl alkyl amines with aroyl chlorides and arylsulfonyl chlorides. The reaction yields ranged from moderate to excellent. Three prepared compounds were found to possess micromolar anti-HSL properties, and therefore can be used as leads for subsequent optimization.

The optimized structures are subsequently tested *in vitro* to determine their potencies. Three compounds **10**, **31**, and **38** were found to be as the most potent synthesized compounds, of anti-HSL with IC_{50} = 10, 37 and 10 µM, respectively.

1. Introduction

1.1 Hormone Sensitive Lipase

In recent years, we have witnessed a flurry of new oral drugs for the treatment of type 2 diabetes. The impetus for developing new diabetes drugs comes from the unmet need for pharmacologic tools that allow diabetic patients to achieve recommended glucose control targets more effectively and safely.

Approximately, 95 % of all patients with diabetes have type 2 insulin-resistant diabetes mellitus. Diabetes is currently the fifth leading cause of death in the United States and is associated with excess morbidity stemming from cardiovascular disease, kidney failure, blindness, and lower limb amputation (Hogan *et al*., 2003). With such rising in the diabetes prevalence, new therapies that provide glucose control are needed.

Elevated plasma levels of free fatty acids (FFAs) are thought to play a major role in the pathogenesis of insulin resistance and type 2 diabetes by inhibiting glucose uptake and utilization by the muscles. The central role of the intracellular enzyme hormone-sensitive lipase (HSL) in regulating fatty acid metabolism makes it an interesting pharmacological target for the treatment of insulin resistant and dyslipidemic disorders where a decrease in delivery of fatty acids to the circulation and thereby reducing insulin resistance is desirable (Ebdrup *et al*., 2004).

1.1.1 Physiological Role of HSL

Free fatty acids are a major energy source for most tissues in mammals. Circulating FFAs in plasma are primarily derived from adipose tissue, which is the main repository for the storage of triglycerides (triacylglycerol). The chief enzyme responsible for the mobilization of FFA from adipose tissue, i.e., lipolysis, is HSL.

HSL is an intracellular, neutral lipase that has broad substrate specificity, catalyzing the hydrolysis of triacylglycerol, diacylglycerol, monoacylglycerol, and cholesteryl esters, as well as retinyl esters. Its activity against diacylglycerol is about 10-fold and 5-fold higher than its activity against triacylglycerol and monoacylglycerol, respectively (Kraemer and Shen, 2006).

Triglyceride (TG) has three ester bonds which can be hydrolyzed, resulting in glycerol and 3FFAs (Figure 1). Although HSL is capable of fully hydrolyzing TG to glycerol and FFA, the enzyme monoacylglycerol lipase is required for an efficient complete hydrolysis of triglycerides (Holm, 2003).

$$CH_3-(CH_2)n\text{-}C(=O)-O-CH_2$$
$$CH_3-(CH_2)n\text{-}C(=O)-O-CH \quad + \quad 3\,H_2O \longrightarrow \quad 3CH_3-(CH_2)nC(=O)-OH \ \text{(Fatty acid)} \quad + \quad \begin{matrix} HO-CH_2 \\ HO-CH \\ HO-CH_3 \end{matrix} \ \text{(Glycerol)}$$
$$CH_3-(CH_2)n\text{-}C(=O)-O-CH_3$$

Figure 1: Triacylglycerol consists of a glycerol backbone and three lipid residues. The bond can be hydrolyzed to produce free fatty acids and glycerol.

As HSL possesses also activity against long chain esters of cholesterol, one would aspect an additional role for HSL in cholesterol metabolism, beside the hydrolysis of lipids. For that reason, HSL is not only present in adipose tissues but is also found in tissues in which cholesterol esters are stored. For instance adrenal cortex, ovaries, testis and heart (Kraemer and Shen, 2006).

1.1.2 Structure of HSL

1.1.2.1 Homology

Most enzymes can be categorized in families. At first, however, HSL did not seem to be a member of known family of mammalian lipases which include lipases as lipoprotein lipase, hepatic lipase and pancreatic lipase (Cordle *et al.,* 1986, Kirchgessner *et al*., 1987). However, a low sequence homology to brefeldin A esterase as well as to a thermophilic carboxylesterase, which both have been solved by X-ray crystallography, was first reported by Contreras (Contreras *et al*., 1996). Contreras constructed a three-dimensional model for the catalytic domain of HSL. On the basis of the sequence homology outlined, models of HSL have been built using the homology modeling program Modeller. The large C-terminal portion and the major part of the regulatory domain of HSL are not included in the model. However, the active site, the oxy-anion hole and large parts of the substrate binding region are expected to be quite reliably modeled. However, due to the low sequence homology and the lack of structural information about the regulatory domain, the predictive value of the model has been limited (Ebdrup *et al*., 2004).

1.1.2.2 Gene Structure

To date three isoforms of HSL have been identified, ranging in size from 84 to 130 kDa. These arise from the same gene through the use of alternative promoters. The human HSL gene is composed of 9 Exons encode the amino acids that are common to all isoforms (Figure 2) (Holm, 2003). Exon 6 contains the catalytic site serine motif found in almost all lipases. This serine is thought to be part of a catalytic triad found in many lipases together with Asp703 and His733. Exons 7 and 8 contain the serine

phosphorylation sites. Exon 9 could contain the lipid binding region as it encodes a hydrophobic stretch (Contreras *et al*., 1996).

In addition to these, several upstream non-coding and coding exons appear to be used in a mutually exclusive and tissue-specific manner (Figure 2). Two promoters have been identified and characterized, i.e. the promoters upstream of exons B and T. The promoter upstream of exon B appears to be utilized in adipocytes as well as several other tissues, whereas the promoter upstream of exon T drives expression specifically in testis (Holm, 2003).

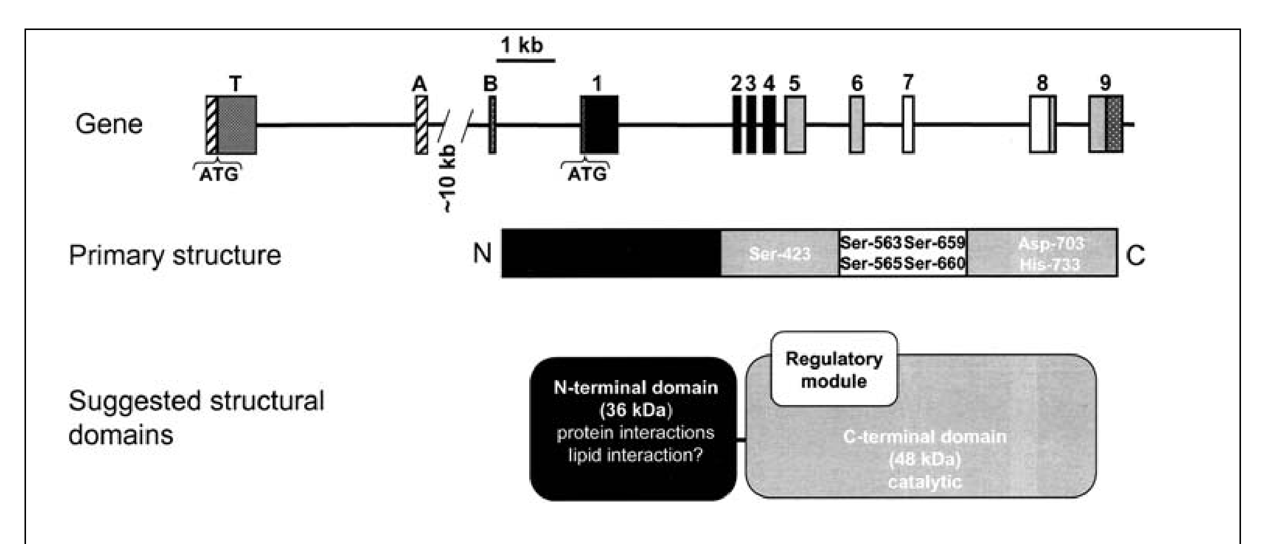


Figure 2: Proposed domain structure of adipocyte HSL
Top: exon/intron organization of the human HSL gene. Middle: linear representation of the primary structure of HSL (numbering from rat HSL). Bottom: domain structure model of adipocyte HSL.
A non-coding exon (B) and nine coding exons (1–9) are represented in the majority of adipocyte HSL transcripts. Exon T is a testis-specific exon, whereas exon A is represented in a minor fraction of adipocyte HSL transcripts. Exons 1–4 encode the N-terminal domain, whereas exons 5–9 encode the catalytic domain. Ser-423, Asp-703 and His-733 are the catalytic triad residues. Exon 7 and part of exon 8 encode the regulatory module, which contains all known phosphorylation sites of HSL.

1.1.2.3 Protein Structure

The knowledge about HSL is still premature but is growing rapidly. A domain structure model for the adipocyte isoform of HSL has been worked out using limited proteolysis studies, spectroscopic analyses during denaturation, molecular modeling and site-directed mutagenesis. According to this, adipocyte HSL is composed of two major structural domains, an N-terminal domain, which is variable between isoforms, and a C-terminal catalytic domain, which is identical in all known HSL isoforms (Figure 2). The function of the N-terminal domain is poorly understood but it has been implicated in protein–protein and protein–lipid interactions. The catalytic domain harbors the active site, including the residues of the catalytic triad, as well as a regulatory module with all known phosphorylation sites of HSL (Holm, 2003).

A three-dimensional model of the catalytic domain of HSL has been built by Contreras (Figure 3), it consists of various α helices and β sheets folded in a so called α/β hydrolase fold, a central β sheet surrounded by a variable number of α helices. Two large differences with homologous enzymes were found. One is the connection between β7 and β8, the other is the connection between β6 and β7. The latter has a major insert containing the phosphorylation sites (Contreras *et al*., 1996).

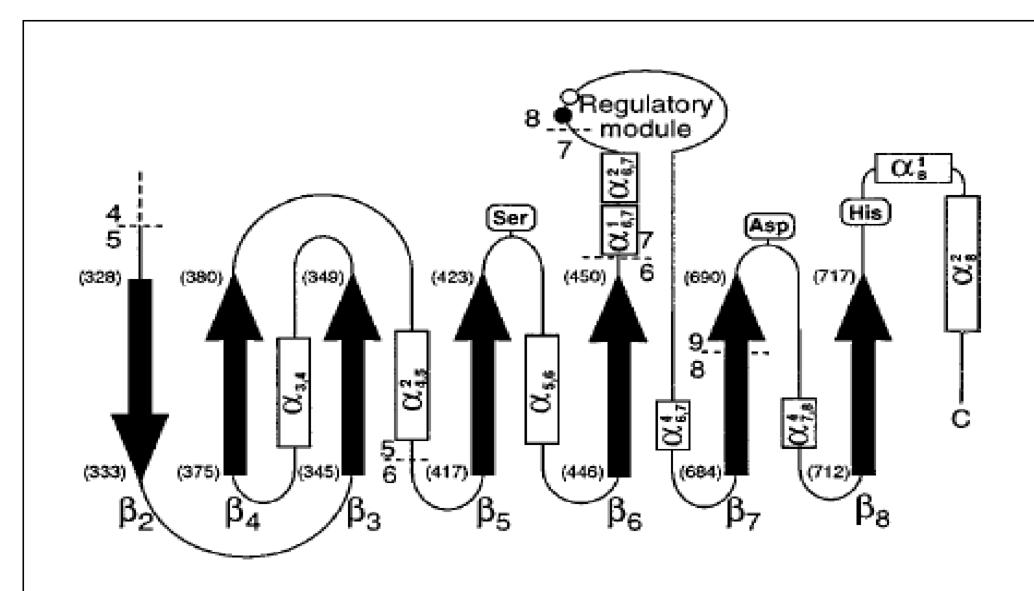


Figure 3.A: Schematic representation of the catalytic domain of HSL.
The exon limits are indicated with dashed lines and the corresponding exons numbers. Numbers in parentheses indicate the residue position (in the human HSL sequence) of the N- and C-terminal residues of each β-strand. The region containing the phosphorylation sites protrudes from the protein. The regulatory site and basal site are shown with circles.

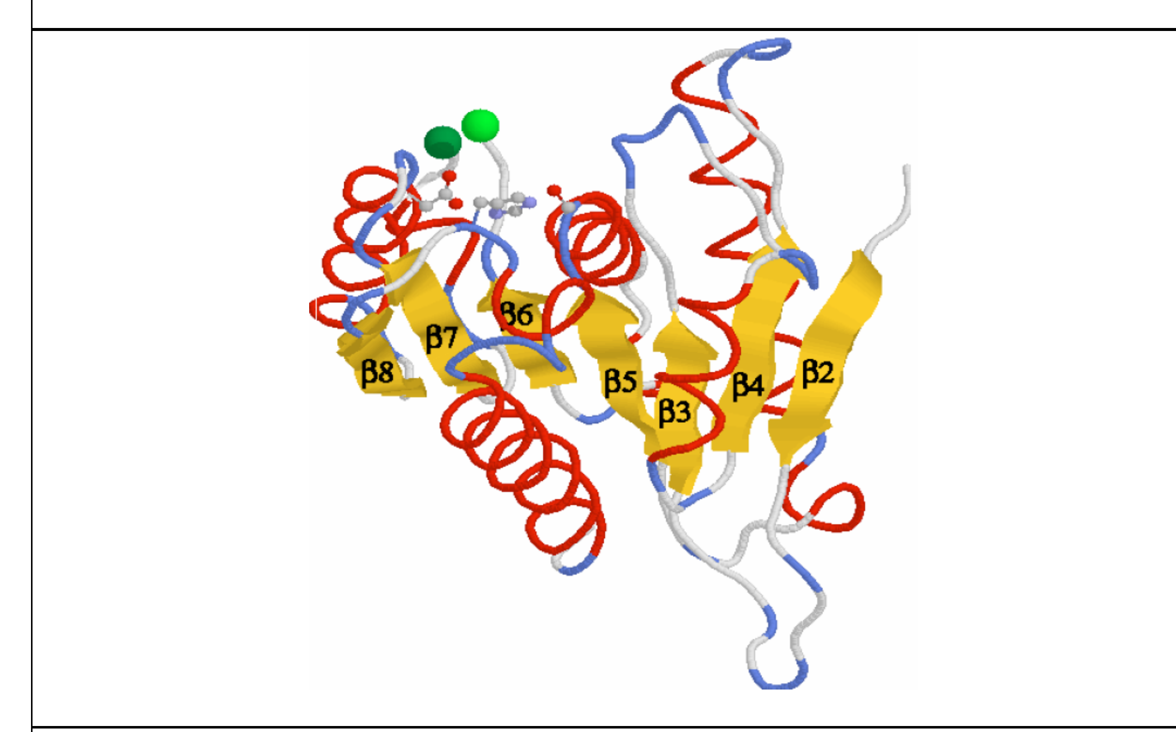


Figure 3.B: Ribbon representation of the model for the catalytic domain of HSL. The strands of the central β sheet are numbered accordingly to the enzymes of the carboxylesterase B family. The residues of the catalytic triad are shown in ball and stick representation. The model does not include a vast area located immediately behind the catalytic triad, inserted in the primary sequence between β strands 6 and 7, that constitues a regulatory module. The N- and C-terminal residues of this regulatory module are indicated by a light green and dark green sphere, respectively.

1.1.3 Regulation and Phosphorylation of HSL

1.1.3.1 Lipolysis

The regulation of lipolysis is complex and, although not completely understood, involves multiple mechanisms, including lipolytic (ACTH, β adrenergic agonists) and anti-lipolytic (insulin) hormones and their cognate receptors and signaling pathways, particularly involving cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA). Current working models for the mechanisms underlying lipolysis have focused on steps downstream of hormone receptors and signaling cascades, concentrating on lipid droplet-associated proteins, such as perilipins, and lipases, such as HSL and others, that appear to play vital roles in lipolysis. In a simplified view (Figure 4), these models suggest that, under basal, unstimulated conditions, perilipin decorates the surface of the lipid droplet, protecting the lipid droplet from hydrolysis by HSL, which is primarily located within the cytosol. Upon lipolytic stimulation, PKA is activated, resulting in the phosphorylation of both perilipin and HSL. Phosphorylation of perilipin then facilitates the translocation of HSL from the cytosol to the lipid droplet, where hydrolysis of triacylglycerol and lipolysis can proceed (Kraemer and Shen, 2006).

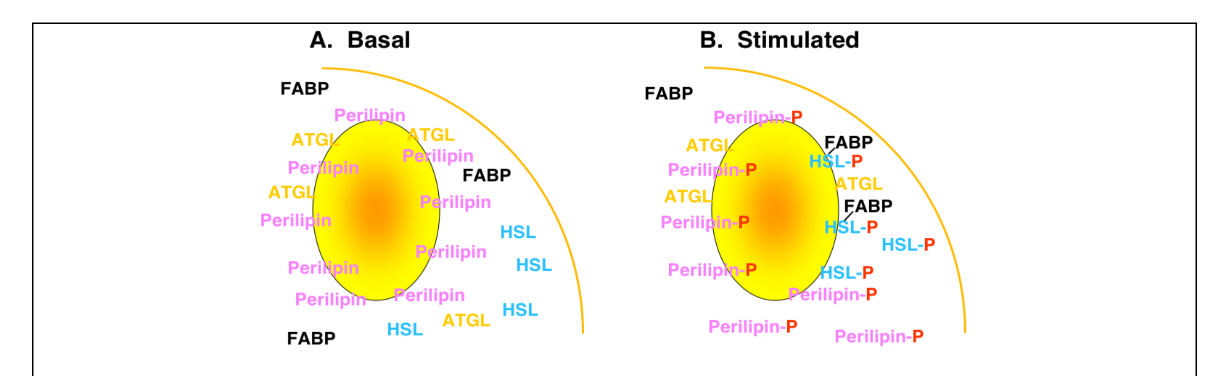


Figure 4: A model of lipolysis Process. Under basal conditions perilipin is localized to the lipid droplet, along with other droplet associated proteins, such as ATGL (adipose triglyceride lipase), whereas HSL is primarily localized in the cytosol along with other cytosolic proteins such as FABP (fatty acid binding protein). Following lipolytic stimulation, PKA is activated, resulting in the phosphorylation of perilipin and HSL. This is associated with the translocation of HSL from the cytosol to the lipid droplet where hydrolysis of the triacylglycerol lipid droplet occurs.

1.1.3.2 Mechanisms behind PKA-Mediated Activation of Lipolysis

Adipocyte lipolysis is regulated by hormones, neurotransmitters and other effector molecules and HSL is one of the major targets of this regulation. Catecholamines are important stimulators of lipolysis, whereas insulin is believed to be the most important anti-lipolytic hormone (Figure 5). Binding of agonists to the β-adrenergic receptors, coupled to adenylate cyclase via the stimulatory G-protein, leads to an increased production of cAMP and activation of PKA. The two main targets for PKA phosphorylation are HSL and the perilipins, which are proteins covering the lipid droplets of adipocytes. PKA phosphorylation of HSL and the perilipins causes a dramatic increase in lipolysis. The ability of insulin to antagonize hormone-induced lipolysis is to a large extent accounted for by its ability to lower cAMP levels and therefore PKA activity. The decrease in cAMP is mainly the result of an insulin-mediated activation of phosphodiesterase 3B (Holm, 2003)..

PKA phosphorylates HSL at three serine residues: 563, 659 and 660 (numbering for rat HSL). *In vitro* PKA phosphorylation of HSL can be monitored as an increased activity against triglyceride and cholesteryl ester substrates. *In vivo*, PKA phosphorylation is known to result in translocation of HSL from a cytosolic location to a location at the lipid droplet. For a long time Ser-563 was believed to be the only PKA site of HSL, and thus the site that confers activity control. However, the observation that mutagenesis of Ser-563 did not abolish the ability of HSL to become activated upon phosphorylation by PKA, led to the identification of two novel PKA sites, Ser-659 and Ser-660. All three PKA sites of HSL are phosphorylated both *in vivo* in response to stimulation of rat adipocytes by isoprenaline and *in vitro* upon incubation with PKA. Mutagenesis of both Ser-659 and Ser-660 completely abolishes activation of HSL *in vitro* as well as translocation *in vivo*, whereas mutagenesis of Ser-563 has no effect on either of these.

Thus Ser-659 and Ser-660 are the activity-controlling sites of HSL, whereas the role of the third PKA site (Ser-563) remains elusive (Holm, 2003).

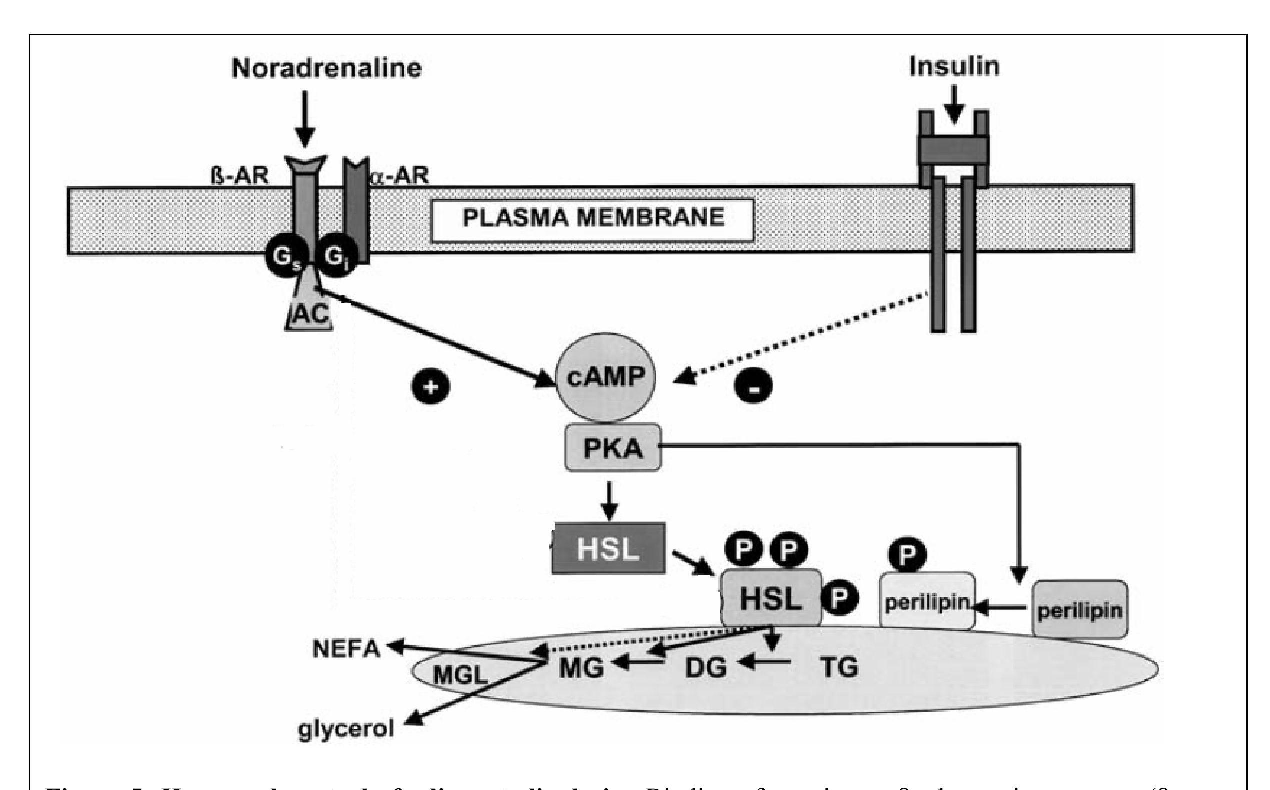


Figure 5: Hormonal control of adipocyte lipolysis. Binding of agonists to β-adrenergic receptors (β-AR), coupled to the adenylate cyclase (AC) via the stimulatory G-protein (Gs), increases the levels of cAMP. This in turn leads to activation of PKA, which phosphorylates HSL at three serine residues and also perilipin A on multiple sites. PKA phosphorylation of HSL causes translocation from the cytosol to the lipid droplet, whereas phosphorylation of perilipin by PKA alleviates the barrier function of this protein and prompts its active participation in the lipolytic process. The anti-lipolytic action of insulin can to a large extent be accounted for by its ability to lower cAMP levels. This in turn is mainly the result of an insulin-mediated activation of phosphodiesterase 3B. TG, triglycerides; DG, diglycerides; MG, monoglycerides; MGL, monoglyceride lipase.

1.1.4 Role of HSL in Diabetes Mellitus

In type 2 diabetes, hyperglycemia is accompanied by abnormalities in lipid metabolism. The elevation of circulating FFAs, in particular, has received much attention and is widely considered a possible pathogenetic factor in the disease. Increased influx of FFAs leads to peripheral insulin resistance seen as decreased glucose uptake in muscle and increased hepatic glucose production (Miles and Nelson, 2007). The mechanism for this effect has been attributed to glucolipotoxia. Furthermore, increased FFAs flux to the liver results in enhanced triglyceride synthesis and assembly into secreted very low density lipoprotein (VLDL), contributing to the abnormal lipoprotein profile seen in diabetics and insulin resistant individuals (Frayn, 2002).

The driving force behind the increased flux of plasma FFAs is an overflow, in adipose tissue, of fatty acids derived from increased intracellular lipolysis and from hydrolysis of chylomicron triglycerides of direct dietary origin. The key rate limiting enzyme in regulating lipolysis in adipose tissue is HSL (De Jong *et al*., 2004).

HSL is a component of the metabolic switch between the use of glucose or FFAs as energy sources. Adipose HSL activity is normally inhibited by insulin, and is thus elevated in hypoinsulinemic states such as fasting. However, HSL remains active in type 2 diabetes, despite elevated insulin levels, presumably through loss of insulin's inhibitory effect. The resulting fatty acid flux stimulates inappropriate hepatic gluconeogenesis (Lowe *et al*., 2004). High FFA levels are further suspected to play a role in the (still unclear) mechanisms of insulin resistance itself (Jianping, 2007). Inhibition of lipolysis through the inhibition of HSL thus has therapeutic implications for type 2 diabetes, with the potential to treat hyperglycemia, dyslipidemia, and possibly their underlying metabolic defects (Lowe *et al*., 2004).

1.1.5 Role of HSL in Obesity

Obesity is a major risk factor for the development of chronic disorders, such as hypertension, type 2 diabetes, dyslipidemia and atherosclerosis, which in turn may cause ischemic heart disease, stroke and premature death. Alterations in free fatty acid metabolism may play an important role in the development of metabolic and cardiovascular complications to obesity (Ebdrup *et al*., 2004).

Obesity increases the risk for diabetes up to 90-fold (Anderson *et al*., 2003). Results of the Behavioral Risk Factor Surveillance System indicate that for every 1-kg increase in weight, the risk for diabetes is increased by 9% (Mokdad *et al*., 2000). Therefore, it is not surprising that the prevalence of diabetes in the United States has risen (from 4.9% in 1990 to 7.9% in 2001) in concert with the prevalence of obesity (doubled over t

he previous 30 years), with no reversal of this trend in the foreseeable future (Mokdad *et al*., 2003). In addition to being a risk factor for the development of diabetes, obesity worsens hyperglycemia, hyperinsulinemia, insulin resistance, and dyslipidemia. Obesity is also a risk factor for hypertension, and cardiovascular disease (Maggio and Pi-Sunyer, 2003).

The central role of the intracellular enzyme HSL in regulating fatty acid metabolism makes it an interesting pharmacological target for the treatment of metabolic disorders where a decrease in delivery of fatty acids to the circulation is desirable, (Ebdrup *et al*., 2004).

1.1.6 Design and Synthesis of new HSL inhibitors guided by Pharmacophore Models.

In a previous publication, Taha et al. (Taha et al., 2008) published two successful pharmacophore hypotheses and an associated QSAR model capable of describing ligand binding to HSL. Figure 6 shows the two pharmaocphores and how they map potent

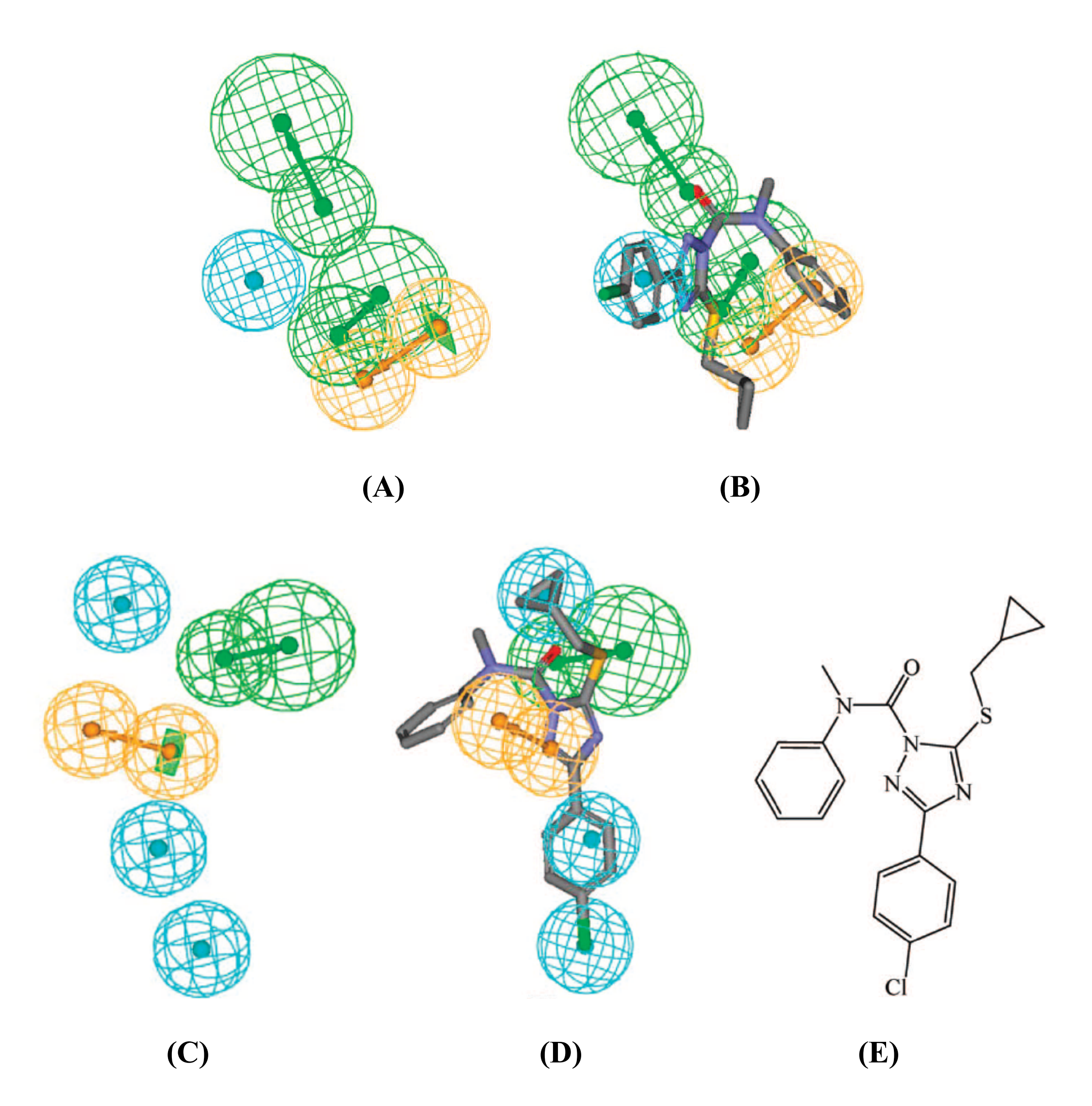


Figure 6: Optimal HSL binding models published by Taha et al. in 2008. **(A)** Binding model Hypo4/9. Features: HBA as green vectored spheres, Hbic as blue spheres, and RingArom as orange vectored spheres; **(B)** Hypo4/9 fitted against a potent HSL inhibitor (IC_{50} = 1.09 nM); **(C)** Hypo8/7; **(D)** Hypo8/7 fitted against the same compound **(E)** The chemical structure of the mapped HSL inhibitor.

HSL.

These models were used as 3D search queries to search for new HSL inhibitors. These efforts culminated in the discovery of several low micromolar and submicromolar HSL inhibitors. Two hits were particularly interesting as they were simple sulfonamide and ester. Figure 7 shows the two compounds and how they map optimal binding models.

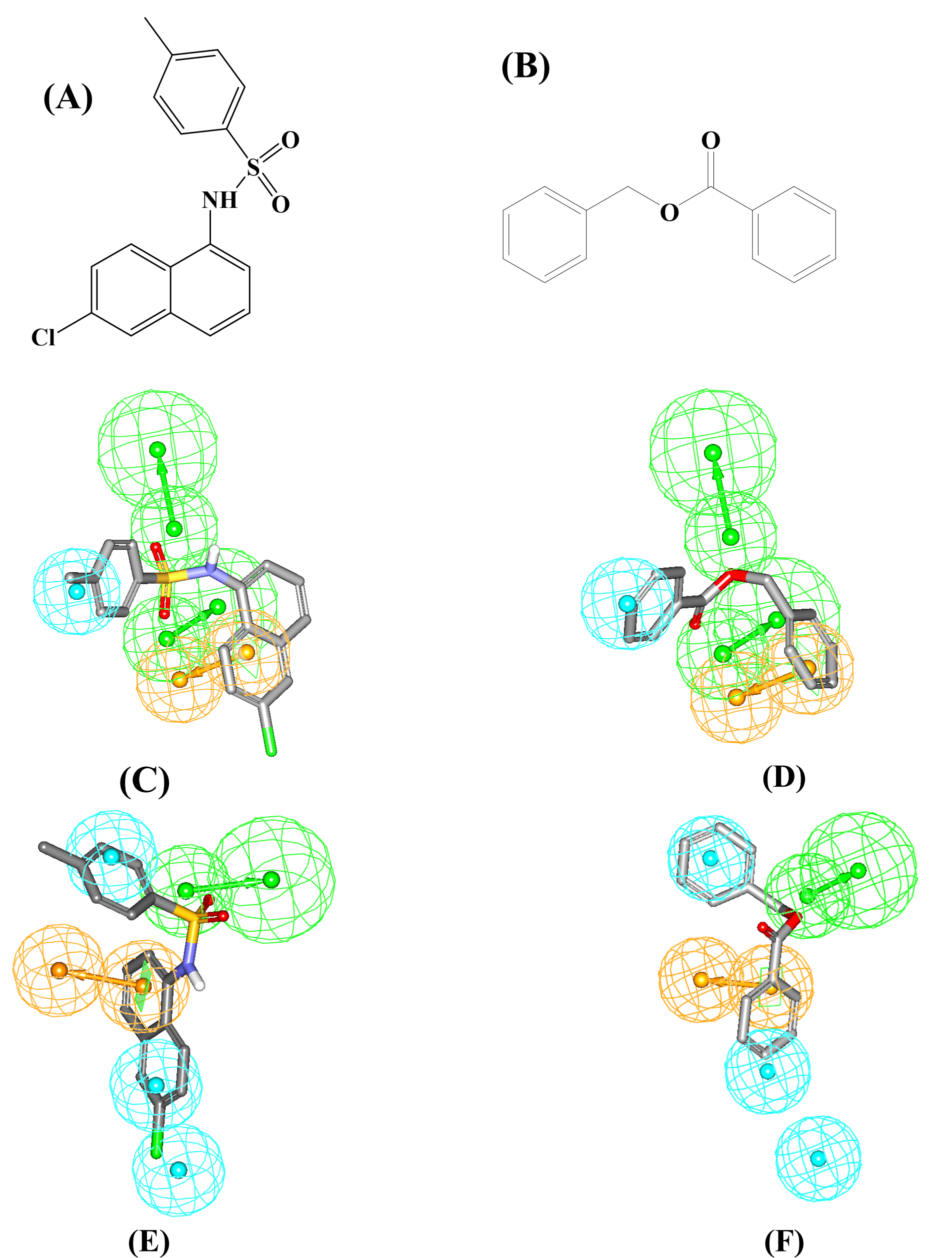


Figure 7: Interesting hits identified by pharmacophore modeling and QSAR analysis followed by in silico screening (Taha *et al.*, 2008). **(A)** ligand Inhibited HSL by 15% at 1μM. **(B)** ligand of anti-HSL IC50 = 1.1 μM. (C), (D), (E) and (F) show how the two hits map Hypo4/9 and Hypo8/7.

The simple structures of the above compounds combined with availability of valid pharmacophore and QSAR models for HSL prompted us to explore potential anti-HSL

properties of a series of esters, amides, sulfonamides and sulfonate esters capable of fitting the two pharmacophore models. Table 1 shows the prepared compounds and their anti-HSL bioacitivities.

2.1. Synthesis of the Optimized Hits

2.1.1 Materials

- Sodium carbonate, Min. 99.5%, Sigma-Aldrich, (USA).
- Triethylamine (TEA), Sigma-Aldrich, (USA).
- Dimethylaminopyridine (DMAP), Sigma-Aldrich, (USA).
- Acetone, Min. 99.5%, was purchased from TEDIA Company (USA).
- Aniline, Min. 99%, From MERCK, (Germany).
- 3-Nitroaniline, From Riedel-de Haën, (Germany).
- 4-Nitroaniline, From Riedel-de Haën (Germany).
- 4-Bromoaniline, Min. 97%, From Aldrich, (USA).
- 4-Fluoroaniline, Min. 97%, From Fluka, (Switzerland).
- 2, 4-Difluoroaniline, From Fluka, (Switzerland).
- 2- Chloroaniline, Min. 98%, From Fluka, (Switzerland).
- 4- Chloroaniline, Min. 98%, From MERCK, (Germany).
- 3, 4-Dichloroaniline, Min. 99%, From Sigma-Aldrich, (USA).
- Phenyl ethylamine, Sigma-Aldrich, (USA).
- *p*- Anisidine, Min. 99%, From Sigma-Aldrich, (USA).
- Benzyl alcohol, 99.8%, From Sigma-Aldrich, (USA).
- 2, 6- Dimethylaniline, Min. 98%, From Fluka, (Switzerland).
- Resorcinol (Benzene -1, 3-diol), 99-100.5%, Laboratory Rasayan.
- 1, 3 -Phenylen diamine, Min. 98%, From MERCK, (Germany).
- Benzyl amine, Min. 99%, From MERCK, (Germany).
- 4- Methoxyaniline, Sigma-Aldrich, (USA).
- 4-Chloro-2-methyl aniline, Sigma-Aldrich, (USA).

- 5- Chloro-2-methyl aniline, Riedel-de Haën, (Germany).
- 2-Methylaniline, Min. 99%, From MERCK, (Germany).
- 3-Methylaniline Min. 99%, From MERCK, (Germany).
- 4- Chloro-2-methyl-phenol, 97%, Riedel-de Haën, (Germany).
- 2-Chloro-4-Nitro-phenol, Min. 97%, From Aldrich, (USA).
- 4-Methylaniline, 99%, From MERCK, (Germany).
- 4- Methylsulfanayl aniline, From Aldrich, (USA).
- 3-Methoxy-4-methyl-aniline,Sigma-Aldrich, (USA)
- 4-Methoxy-aniline (p-anisidine), Min. 98%, From Fluka, (Switzerland).
- 3-Methoxy-aniline (o-anisidine), Min. 98%, From Fluka, (Switzerland).
- Benzoyl chloride, Min. 99%, From MERCK, (Germany).
- 4-Methyl-benzoyl chloride, Min. 98%, Sigma-Aldrich, (USA).
- 4- Nitro-benzoyl chloride, Min. 98%, From MERCK, (Germany).
- 3- Nitro-benzoyl chloride, Min. 98%, From Acros, (USA).
- 3, 5-Dinitro-benzoyl chloride, Min. 99%, From MERCK, (Germany).
- Benzenesulfonyl chloride, Min. 99%, Sigma-Aldrich, (USA).
- Benzenesulfonyl chloride, Min. 99%, From Fluka, (Switzerland).
- 3-Nitro- Benzenesulfonyl chloride, Min. 97%, Sigma-Aldrich, (USA).
- 4-Methyl Benzenesulfonyl chloride, Min. 97%, From Fluka, (Switzerland).
- Pyridine, 99.8%, Sigma-Aldrich, (USA).
- Stuart scientific electro-thermal melting point apparatus.

2.1.2 General Methods

Melting points were measured using Gallenkampf melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectrums were collected on a Bruker NMR 300MHz spectrometer. High resolution mass spectrometry was performed using LC Mass Bruker Apex-IV mass spectrometer utilizing an electrospray interface. Infrared spectra were recorded using Shimadzu IR Affinity-1 spectrophotometer. The samples were analyzed as KBr pellets. Analytical thin layer chromatography (TLC) was carried out using pre-coated aluminum plates and visualized by UV light (at 254 and/or 360 nm). Elemental analysis was performed using EuroVector elemental analyzer. Chemicals and solvents were used without further purification.

2.1.3 General procedures for the preparation of aryl and arylalkyl esters and amide derivatives

Method A

To a magnetically-stirred, ice-bathed, solution of the substituted aniline (or aryl alkyl amine) and triethylamine (2 equiv) in dry acetone (50 mL), benzoyl chloride or sulfonyl chloride derivative (2 equiv) in acetone was added. The solution was stirred at room temperature until completion (as revealed by TLC). The reaction mixture was quenched by slow addition into sufficient 5% aqueous sodium bicarbonate solution to neutralize all generated acid. The precipitated crude amide (or ester) products were purified by recrystallization from acetone/water (Scheme 1 and 2).

Z= H, $N(CH_3)_2$

X=OH, NH_2

Scheme 1: Synthetic route for synthesis of acyl amide or ester.

Z= H, $N(CH_3)_2$

X=OH, NH_2

Scheme 2: Synthetic route for synthesis of acyl sulfonamide or sulfone ester.

Method B

To a magnetically-stirred solution of the substituted aniline (phenol or aryl alkyl amine) in dry pyridine (50 mL), benzoyl chloride (or sulfonylchloride) derivative (1.5 equiv) was added. The solution was refluxed until completion (as revealed by TLC). The reaction mixture was quenched by addition of water (200 mL). The precipitated crude amide and ester products were subsequently washed with methanol (Scheme 1 and 2).

N-Benzyl-4-methyl-benzamide (1)

Prepared as in method A from commercially available 4-Methylbenzoyl chloride (2.31 g, 0.019 mol) dissolved in acetone (50 mL) and benzylamine (1.39 g, 0.0129 mol) to yield title compound as white colored powder (2.58 g, 89%). mp:135°C (lit. mp =135°C (Agwada, 1982)); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.40 (s, 3H), 4.64 (d, 2H, J = 7.2), 6.40 (brs,1H, NH), 7.2-7.3 (m, 7H), 7.67-7.71 (m, 2H) ppm;^{13}C NMR (100 MHz, $CDCl_3$): δ 16.9 (CH_3), 39.6 (CH_2), 122.4 (2xCH), 123.08 (CH), 123.4 (2xCH), 124.3 (2xCH), 124.7 (2xCH), 127.0 (C), 133.8 (C), 137.5 (C), 162.8 (C=O) ppm. Anal. Calcd for $C_{11}H_{10}Cl_2N_4O$: C, 79.97; H, 6.71; N, 6.22; found: C, 79.21; H, 6.54; N, 6.21.

N-(4-Chloro-phenyl)-4-nitro-benzamide (2)

Prepared as in method A from commercially available 4-nitrobenzoyl chloride (3.7 g, 0.019 mol) dissolved in acetone (50 mL) and 4-chloroaniline (1.28g, 0.01 mol) to yield title compound as yellow colored powder (2.69 g, 97%). mp 219°C (lit. mp = 219 °C (Jadhav and Sukhatankar, 1939)); HRMS-ESI m/z $[M-H]^-$ calcd for $C_{13}H_8ClN_2O_3$: 275.02234, found: 275.02229. Anal. Calcd for $C_{13}H_9ClN_2O_3$: C, 56.43; H, 3.28; N, 10.13; found: C, 56.34; H, 3.12; N, 9.99.

4-Nitro-N-(4-nitro-phenyl)-benzamide (3)

Prepared as in method A from commercially available 4-Nitrobenzoyl chloride (3.71 g, 0.019 mol) dissolved in acetone (50 mL) and 4- nitroaniline (1.38 g, 0.01mol) to yield title compound as yellow colored powder (2.1 g, 76%). mp:268-269°C (lit. mp=268.5-269.5°C (Witjens *et al.*, 1943)); HRMS-ESI *m/z* $[M{-}H]^-$ calcd for $C_{13}H_8N_3O_5$: 286.04640, found: 286.04605. Anal. Calcd for $C_{13}H_9N_3O_5$: C, 54.36; H, 3.16; N, 14.63; found: C, 53.88; H, 3.15; N, 14.14.

4-Nitro-benzoic acid 3-hydroxy-phenyl ester (4)

Prepared as in method A from commercially available 4-Nitrobenzoyl chloride (3.7 g, 0.019 mol) dissolved in acetone (50 mL) and Benzene-1, 3-diol (1.05 g, 0.01 mol) to yield title compound as Off white colored powder (2.08 g, 80%). mp:173-175 °C (lit. mp = 175-177°C (Siebenmann *et al.*, 1643)); HRMS-ESI *m/z* $[M{-}H]^-$ calcd for $C_{13}H_8NO_5$: 258.04025, found: 258.04012. Anal. Calcd for $C_{13}H_9NO_5$: C, 60.24; H, 3.50; N, 5.40; found: 59. 97; H, 3.47; N, 5.23.

N-(2, 6-Dimethylphenyl)-4-nitrobenzamide (5)

Prepared as in method A from commercially available 4-Nitrobenzoyl chloride (3.71 g, 0.019 mol) dissolved in acetone (50 mL) and 2, 6-dimethylaniline (1.15 g, 0.01 mol) to yield title compound as white colored powder (1.8g, 70%). mp: 194- 196 °C (lit. mp= 193-196 °C (Adams and Werbel, 1958)); HRMS-ESI *m/z* $[M{-}H]^-$ calcd for $C_{15}H_{13}N_2O_3$: 269.09262, found: 269.09204. Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36; found: C, 65.94; H, 5.21; N, 9.70.

N-(3-Nitrophenyl)-4-nitrobenzamide (6)

Prepared as in method A from commercially available 4-Nitrobenzoyl chloride (3.7 g, 0.019 mol) dissolved in acetone (50 mL) and 3- nitroaniline (1.38 g, 0.01 mol) to yield title compound as yellow colored powder (1.97 g, 72%). mp: 227-228°C (lit. mp=227-228°C (Kang *et al.*, 2008)); HRMS-ESI *m/z* $[M{-}H]^-$ calcd for $C_{13}H_8N_3O_5$: 286.04640, found: 286.04604. Anal. Calcd for $C_{13}H_9N_3O_5$: C, 54.36; H, 3.16; N, 14.63; found: C, 53.91; H, 2.81; N, 14.50.

4-Methyl-N-(3-nitro-phenyl)-benzamide (7)

Prepared as in method A from commercially available 4-Methyl benzoyl chloride (3g, 0.02 mol) dissolved in acetone (50 mL) and 3- nitroaniline (1.38g, 0.01 mmol) to yield title compound as white colored powder (2.07 g, 81%). mp:171-172°C (lit. mp =172°C (Takatori, 1954)); HRMS-ESI *m/z* $[M{-}H]^-$ calcd for $C_{14}H_{11}N_2O_3$: 255.07697, found: 255.07701. Anal. Calcd for $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.72; N, 10.93; found: C, 65.18; H, 4.09; N, 10.38.

N-Benzyl-4-nitro-benzamide (8)

Prepared as in method A from commercially available 4-Nitrobenzoyl chloride (3.7 g, 0.019 mol) dissolved in acetone (50 mL) and Benzylamine (1.39g, 0.0129 mol) to yield title compound as white colored powder (2.187 g, 90%). mp:138-139°C (lit. mp = 138-139 °C (Kokare *et al.*, 2007)); HRMS-ESI *m/z* $[M{-}H]^-$ calcd for $C_{14}H_{11}N_2O_3$: 255.07697, found: 255.07689. Anal. Calcd for $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.72; N, 10.93; found: C, 64.98; H, 4.63; N, 10.28.

N-(4-Bromo-phenyl)-4-methyl-benzenesulfonamide (9)

Prepared as in method A from commercially available 4-methyl Benzene sulfonyl chloride (3.8 g, 10 mmol) dissolved in acetone (50 mL) and 4-bromoaniline (1.71 g, 0.01 mol) to yield title compound as off-white colored powder (2 g, 62%). mp: 87- 88 °C (lit. mp = 87°C from methanol (Hellwinkel and Lenz, 1985)); HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{13}H_{12}BrNNaO_2S$: 347.96698, found: 347.96654. Anal. Calcd for $C_{13}H_{12}BrNO_2S$: C, 47.86; H, 3.71; N, 4.29; found: C, 46. 83; H, 3.78; N, 4.07.

N-(2, 6-Dimethyl-phenyl)-4-methyl-benzamide(10)

Prepared as in method A from commercially available 4-methyl benzoyl chloride (3.1 g, 0.019 mol) dissolved in acetone (50 mL) and 2, 6-dimethylaniline (1.15 g, 0.01 mol) to yield title compound as faint pink colored powder (1. 97 g, 87%). mp: 163-164°C (lit. mp =163-164°C (Beedle and Robertson, 1988)); HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{16}H_{17}NNaO$: 262.12078, found: 262.12033. Anal. Calcd for $C_{16}H_{17}NO_2S$:79.97; H, 6.71; N, 6.22; found: C, 76. 93; H, 6.07; N, 5.89.

N-(2,6-Dimethyl-phenyl)-benzene sulfonamide (11)

Prepared as in method A from commercially available Benzene sulfonyl chloride (3.5g, 0.019mol) dissolved in acetone (50 mL) and 2, 6-dimethylaniline (1.15g, 0.01mol) to yield title compound as faint pink colored powder (2.159 g, 87%). mp: 130-131°C (lit. mp =130°C (Moorthy and Saha, 2009)); HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{14}H_{15}NNaO_2S$: 284.07212, found: 284.07189. Anal. Calcd for $C_{14}H_{15}NO_2S$: C, 64.34; H, 5.79; N, 5.36; found: C, 64.09; H, 5.15; N, 5.17.

N-(2-Chloro-phenyl)-4-nitro-benzamide (12)

Prepared as in method A from commercially available 4-Nitrobenzoyl chloride (3.7 g, 0.019 mol) dissolved in acetone (50 mL) and 2-chloroaniline (1.27 g, 0.01 mol) to yield title compound as white colored powder (1.95 g, 71%). mp:157-158°C (lit. mp = 157-158°C (Bahrami *et al.*, 2009); v_{max} (KBr disc) 3295, 1656, 1592 cm^{-1}; ^1H NMR (300 MHz, DMSO): δ 7.27- 7.41 (m, 2H), 7.54-7.58 (m, 2H), 8.17 (d, 2H, *J*= 8.9), 8.34 (d, 2H, *J*= 8.8), 10.43 (brs, 1H, NH) ppm; HRMS-ESI *m/z* $[M-H]^-$ calcd for $C_{13}H_8ClN_2O_3$: 275.02234, found: 275.02185. Anal. Calcd for $C_{13}H_9ClN_2O_3$: C, 56.43; H, 3.28; N, 10.13; found: C, 56.03; H, 3.24; N, 9.75.

N-(2, 6-Dimethyl-phenyl)-benzamide (13)

Prepared as in method A from commercially available benzoyl chloride (2.8 g, 0.019 mol) dissolved in acetone (50 mL) and 2, 6-dimethylaniline (1.15 g, 0.01 mol) to yield title compound as white colored powder (1.99 g, 93%). mp:171°C (lit. mp =171-173°C (Da Settimo *et al.*, 2008)) ; HRMS-ESI *m/z* $[M{-}H]^-$ calcd for $C_{15}H_{14}NO$: 224.10754, found: 224.10747. Anal. Calcd for $C_{15}H_{15}NO$: C, 79.97; H, 6.71; N, 6.22; found: C, 78.81; H, 6.34; N, 5.55.

N-(4-Methoxy-phenyl)-4-methyl-benzamide (14)

Prepared as in method A from commercially available 4-Methyl benzoyl chloride (3.1g, 0.0194mol) dissolved in acetone (50 mL) and 4-Methoxyphenylamine (1.169g, 0.01mol) to yield title compound as white colored powder (1.99 g, 87%). mp:72-73 °C (lit. mp = 71-73°C from dichloromethane (Katritzky *et al.*, 2004); HRMS-ESI *m/z* $[M{-}H]^-$ calcd for $C_{15}H_{14}NO_2$: 240.10245, found: 240.10221. Anal. Calcd for $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.81; found: C, 74.43; H, 5.82; N, 5.54.

N-o-Tolyl-benzenesulfonamide (15)

Prepared as in method A from commercially available Benzene sulfonyl chloride (3.5 g, 0.02 mol) dissolved in acetone (50 mL) and 2-Methylaniline (1.07 g, 0.01 mol) to yield title compound as white colored powder (1.95 g, 79%). mp:76-77°C (lit. mp =77°C (Hellwinkel and Lenz, 1985); HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{13}H_{13}NaNO_2S$: 270.05647, found: 270.05587. Anal. Calcd for $C_{13}H_{13}NO_2S$: C, 63.13; H, 5.30; N, 5.66; found: C, 62.83; H, 4.98; N, 5.14.

N-*m*-Tolyl-benzenesulfonamide (16)

Prepared as in method A from commercially available Benzene sulfonyl chloride (3.5 g, 0.02 mol) dissolved in acetone (50 mL) and 3-Methylaniline (1.07 g, 0.01mol) to yield title compound as white colored powder (2.1 g, 85%). mp: 95°C (lit. mp = 94-95°C (Hosseinzadeh *et al.,* 2010)); HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{13}H_{13}NaNO_2S$: 270.05647, found: 270.05587. Anal. Calcd for $C_{13}H_{13}NO_2S$: C, 63.13; H, 5.30; N, 5.66; found: C, 62.85; H, 5.21; N, 5.35.

Benzoic acid 3-hydroxy-phenyl ester (17)

Prepared as in method A from commercially available benzoyl chloride (2.1 g, 0.015 mol) dissolved in acetone (50 mL) and Benzene-1, 3-diol (1.1 g, 0.01mol) to yield title compound as white colored powder (1.88 g, 88 %). mp:131-132 °C (lit. mp = 131-133°C (Bell, 1987)); HRMS-ESI *m/z* $[M{-}H]^-$ calcd for : 213.05517, found:213.05506. Anal. Calcd for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71; found: C,71.22; H, 4.08.

N-(4-Bromo-phenyl)-3, 5-dinitro-benzamidemethane (18)

Prepared as in method B from commercially available 3, 5-dinitrobenzoyl chloride (2.34 g, 0.015 mol) dissolved in pyridine (50 mL) and 4-bromoaniline (1.1 g, 0.01 mol) to yield title compound as white colored powder (3.56 gm, 93%). mp:250-252°C (lit. mp = 251°C (van Horssen, 1936)); HRMS-ESI *m/z* $[M{-}H]^-$ calcd for $C_{13}H_7BrN_3O_5$: 363.95691, found: 363.95661. Anal. Calcd for $C_{13}H_8BrN_3O_5$: C, 44.00; H, 3.16; N, 11.00; found: C, 43.59; H, 3.08; N, 11.10.

4-Methyl-benzoic acid benzyl ester (19)

Prepared as in method A from commercially available 4-Methylbenzoyl chloride (3 g, 0.019mol) dissolved in acetone (50 mL) and Phenylmethanol (1.08 g, 0.01mol) to yield title compound as white colored powder (2.01 g, 89%). mp:46-47°C (lit. mp= 46-47°C (Rueggeberg *et al., 1945*)). ^{1}H NMR (300 MHz, DMSO): δ 2.3(s, 3H), 4.3(s, 2H), 7.13 (d, 2H, J =1.9), 7.22-7.83 (m, 5H), 7.9 (d, 2H, J = 2) ppm; Anal. Calcd for $C_{15}H_{14}O_2$: C, 79.62; H, 6.24; found: C, 79.21; H, 6.14.

4-Methyl-N-phenethyl-benzamide (20)

Prepared as in method A from commercially available 4-Methylbenzoyl chloride (3 g, 0.019 mol) dissolved in acetone (50 mL) and Phenethyl amine (1.2 g, 0.01 mol) to yield title compound as white colored powder (2.12 g, 89%). mp:85°C (lit. mp =84-86°C (Xing *et al*., 2009)); HRMS-ESI *m/z* $[M-H]^-$ calcd for $C_{16}H_{16}NO$: 238.12319, found: 238.12286. Anal. Calcd for $C_{16}H_{17}NO$: C, 80.30; H, 7.16; N, 5.85; found: C, 79.91; H, 6.54; N, 5.71.

N-(4-Fluoro-phenyl)-4-nitro-benzamide (21, Pews, 1971**)**

Prepared as in method A from commercially available 4-nitrobenzoyl chloride (3.7 g, 0.019 mol) dissolved in acetone (50 mL) and 4-fluoroaniline (1.28 g, 0.01mol) to yield title compound as white colored powder (2 g, 77%). mp:173-174°C; ν_{max} (KBr disc) 3305, 1651, 1604, 1547cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 7.15-7.21 (m, 2H), 7.74-7.79 (m, 2H), 8.13 (d, 2H, J = 8.9), 8.31(d, 2H, J = 8.9), 10.58 (brs,1H, NH)ppm; ^{13}C NMR (75.48 MHz, DMSO): δ 115.7 (2xCH, J = 22), 122.8 (2xCH, J = 8), 124 (2xCH), 129.9 (2xCH), 135.5 (C, J = 404.95, C-F), 140.9 (C), 149.7 (C), 157.5 (C, J = 2, C-F), 164.3 (C=O) ppm. HRMS-ESI m/z [M−H]$^{-}$ calcd for $C_{13}H_8FN_2O_3$: 259.05190, found:259.05115. Anal. Calcd for $C_{13}H_9FN_2O_3$: C, 60.00; H, 3.49; N, 10.77; found: C, 59.26; H, 3.54; N, 10.41.

N-Phenyl-benzamide (22)

Prepared as in method A from commercially available benzoyl chloride (5.6 g, 0.039 mol) dissolved in acetone (50 mL) and aniline (1.86 g, 0.02mol) to yield title compound as white colored powder (3.578 gm, 91%). mp:161-162°C (lit. mp =161-162°C (Burtner and Brown, 1953)) ; HRMS-ESI m/z [M−H]$^{-}$ calcd for $C_{13}H_9NO$: 196.07624, found: 196.07599. Anal. Calcd for $C_{13}H_{10}NO$: C, 79.16; H, 5.62; N, 7.10; found: C, 78.20; H, 5.35; N, 6.87.

N-(4- Bromophenyl) –benzamide (23)

Prepared as in method A from commercially available benzoyl chloride (2.8 g, 0.019 mol) dissolved in acetone (50 mL) and 4-bromoaniline (1.7 g, 0.01mol) to yield title compound as white colored powder (2.06 gm, 75%). mp:198-199°C (lit. mp=198-199°C (Hurd and Hayao, 1954)); HRMS-ESI *m/z* $[M-H]^-$ calcd for $C_{13}H_9BrNO$: 273.98675, found: 273.98667. Anal. Calcd for $C_{13}H_{10}BrNO$: C, 56.55; H, 3.65; N, 5.07; found: C, 55.74; H, 3.57; N, 4.77.

N-(3-Amino-phenyl)-benzamide (24)

Prepared as in method A from commercially available benzoyl chloride (2.8 g, 0.019 mol) dissolved in acetone (50 mL) and Benzene-1, 3-diamine (1.08g, 0.01mol) to yield title compound as white colored powder (1.45 gm, 68 %). mp:123-124°C (lit. mp=123-125°C (Thiel *et al.*, 1986)); HRMS-ESI m/z $[M+H]^+$ calcd for $C_{13}H_{13}N_2O$: 213.10279, found: 213.10224. Anal. Calcd for $C_{13}H_{12}N_2O$: C, 73.56; H, 5.70; N, 13.20; found: C, 72.62; H, 4.95; N, 12.83.

N-(3-Nitrophenyl)-benzamide (25)

Prepared as in method A from commercially available benzoyl chloride (2.8 g, 0.019 mol) dissolved in acetone (50 mL) and 3-Nitroaniline(1.38 g, 0.01 mol) to yield title compound as light yellow crystalline powder (2.25gm, 93%). mp: 157°C (lit. mp =157°C (Chattopadhyay *et al.*, 2008)) ; HRMS-ESI m/z $[M-H]^-$ calcd for $C_{13}H_9N_2O_3$: 241.06132, found: 241.06116. Anal. Calcd for $C_{13}H_{10}N_2O_3$: C, 64.46; H, 4.16; N, 11.56; found: C, 63.57; H, 4.09; N, 10.86.

N-(4-Nitrophenyl)-benzamide (26)

Prepared as in method A from commercially available benzoyl chloride (2.8 g, 0.019mol) dissolved in acetone (50 mL) and 4-Nitroaniline(1.38 g, 0.01 mol) to yield title compound as light yellow crystalline powder (2.285 g, 94%). mp: 159-161°C (lit. mp =160-162°C (Roice *et al.,* 2004)) ; HRMS-ESI m/z $[M-H]^-$ calcd for $C_{13}H_9N_2O_3$: 241.06132, found: 241.06116. Anal. Calcd for $C_{13}H_{10}N_2O_3$: C, 64.46; H, 4.16; N, 11.56; found: C, 63.76; H, 4.10; N, 10.98.

N-(3-Amino-phenyl)-4-methyl-benzamide (27)

Prepared as in method A from commercially available 4-Methylbenzoyl chloride (3.1 g, 0.019 mol) dissolved in acetone (50 mL) and Benzene-1,3-diamine (1.08 g, 0.01mol) to

yield title compound as white crystalline powder (1.38 g, 61%). mp: 146 °C (lit. Mp=146°C (Al-Nadaf *et al.*, 2010)). HRMS-ESI m/z $[M+H]^+$ calcd for $C_{14}H_{15}N_2O$: 228.12179, found: 228.12124. Anal. Calcd for $C_{14}H_{14}N_2O$: C, 74.31; H, 6.24; N, 12.38; found: C, 73.84; H, 6.18; N,11.89.

N-(4-Methoxy-phenyl)-3-nitro-benzamide (28)

Prepared as in method B from commercially available 3-Nitrobenzoyl chloride (10.58g, 0.057 mol) dissolved in pyridine (50 mL) and 4-Methoxy-phenylamine (4.87g, 0.038 mol) to yield title compound as yellowish-green crystalline powder (8.78g, 85%). mp: 197-198 °C (lit. mp=196-198°C (Serdons *et al.*, 2009)). HRMS-ESI m/z $[M-H]^-$ calcd for $C_{14}H_{11}N_2O_4$: 271.07188, found: 271.07143. Anal. Calcd for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.44; N, 10.29; found: C, 60.21; H, 3.90; N,9.99.

N-(5-Chloro-2-methyl-phenyl)-benzamide (29)

Prepared as in method A from commercially available benzoyl chloride (2.8 g, 0.019mol) dissolved in acetone (50 mL) and 5-Chloro-2-methylphenylamine (1.42 g, 0.01mol) to yield title compound as white colored powder (1.88 gm, 77%). mp: 143-144°C (lit. mp = 143-143.5°C (Schwartz and Skaptason, 1966)) ; HRMS-ESI m/z

$[M-H]^-$ calcd for $C_{14}H_{11}ClNO$: 244.05292, found: 244.05287. Anal. Calcd for $C_{14}H_{12}ClNO$: C, 68.44; H, 4.92; N, 5.70; found: C, 68.20; H, 4.05; N, 5.42.

N-p-Tolyl-benzenesulfonamide (30)

Prepared as in method A from commercially available Benzene sulfonyl chloride (3.5 g, 0.019 mol) dissolved in acetone (50 mL) and 4-methylaniline (1 g, 0.01mol) to yield title compound as white crystalline powder (2.29 g, 93%). mp:119-120°C (lit. mp = 118-120°C (Hosseinzadeh *et al.,* 2010)); ^{1}H NMR (300 MHz, DMSO): δ 2.34 (s, 3H), 6.98 (d, 2H, J = 8.7), 7.1 (d, 2H, J = 8.7), 7.51-7.71 (m, 5H), 10.19 (brs,1H, NH) ppm;^{13}C NMR (75.48 MHz, DMSO): δ 15.55 (CH_3), 121.7 (2xCH), 127.1 (2xCH), 127.8 (2xCH), 129.8 (2xCH), 133.4 (CH), 133.9(C), 135.2 (C), 139.7 (C)ppm. Anal. Calcd for $C_{13}H_{13}NO_2S$: C, 63.13; H, 5.30; N, 5.66; found: C, 62.45; H, 5.32; N, 5.28.

N-(3-Methoxy-4-methyl-phenyl)-benzamide (**31**, Berneth**,** 1986)

Prepared as in method A from commercially available benzoyl chloride (2.8 g, 0.019 mol) dissolved in acetone (50 mL) and 3-Methoxy-4-methylaniline (1.4 g, 0.01mol) to yield title compound as white colored powder (1.75 gm, 73%). mp: 143-144°C; ν_{max} (KBr disc) 3241, 2930, 2843, 1650, 1610 cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 2.14 (s, 3H), 3.36 (s, 3H), 6.71 (d, 1H, J = 8.34), 6.86 (d, 1H, J = 8.34), 6.9 (d, 1H, J =1.9), 7.45-7.67(m, 5H), 9.35 (brs, 1H, NH) ppm;^{13}C NMR (75.46 MHz, DMSO): δ 20.67

(CH_3), 55.9 (O-CH_3), 112.2 (CH), 125.4 (C), 126.5 (CH), 127.1 (2xCH), 127.5 (CH), 129.2 (2xCH), 129. 7 (C), 132.9 (CH), 141.1 (C), 150.8 (C), 165.4 (C=O)ppm; HRMS-ESI *m/z* $[M-H]^-$ calcd for $C_{15}H_{14}NO_3$: 240.10245, found: 240.10189. Anal. Calcd for $C_{15}H_{15}NO_3$: C, 74.67; H, 6.27; N, 5.81; found: C, 74.18; H, 5.98; N, 5.79.

Toluene-4-sulfonic acid 2-chloro-4-nitro-phenyl ester (32)

Prepared as in method A from commercially available 4-methyl Benzene sulfonyl chloride (3.8 g , 0.019 mol) dissolved in acetone (50 mL) and 2-Chloro-4-nitrophenol (1.73 g, 0.01mol) to yield title compound as brown colored powder (2.72g, 83%). mp:106-107°C (lit. mp = 106-107°C (Suttle and Williams, 1983)); HRMS-ESI *m/z* $[M+Na]^+$ calcd for $C_{13}H_{10}ClNaNO_5S$: 349.98659, found:349.78654. Anal. Calcd for $C_{13}H_{10}ClNO_5S$: C, 47.64; H, 3.08; N, 4.27; found: C, 46. 93; H, 2.89; N, 4.26.

N-(2, 4-Difluoro-phenyl)-benzenesulfonamide (33, Sibi and Lichter, 1980**)**

Prepared as in method A from commercially available sulfonyl chloride (3.5 g, 0.019 mol) dissolved in acetone (50 mL) and 2, 4-difluoroaniline (1.29gm, 0.01mol) to yield title compound as brown colored powder (2 g, 71%). mp: 173-174°C; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{12}H_{11}F_2NO_2S$: 270.04003, found: 270.04000. ν_{max} (KBr disc) 3291,

1597 cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 6.95-7.02 (m, 1H), 7.1-7.2 (m, 2H), 7.3 (d, 2H, J = 8.1), 7.5 (d, 2H, J = 8.1), 7.68 (brs,1H, NH)ppm; Anal. Calcd for $C_{12}H_9F_2NO_2S$: C, 53.53; H, 3.37; N, 5.20; found: C, 53.53; H, 3.47; N, 4.78.

N-(2, 4-Difluoro-phenyl)-benzamide (34, Chen *et al.,* 2005**)**

Prepared as in method A from commercially available 4-methyl benzoyl chloride (3.1 g, 0.02 mol) dissolved in acetone (50 mL) and 2, 4-difluoroaniline (1.29 g, 0.01mol)to yield title compound as white colored powder (1.88 gm, 77%). mp: 131-132°C; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{14}H_{10}F_2NO$: 246.07304, found: 246.07279. ν_{max} (KBr disc) 3265, 1644, 1604 cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 2.94 (s, 3H), 6.5 (d, 2H, J = 7), 7.2-7.59 (m, 3H), 7.66 (d, 2H. J = 7), 8.3 (brs,1H, NH)ppm; ^{13}C NMR (75.48 MHz, DMSO): δ 21.5 (CH_3), 104.5 (CH), 111.4 (CH), 122.8(C), 128.3(2xCH), 128.9(C), 129.5 (2xCH), 130.4 (C), 142.4(CH), 158.2 (C), 161.7(C), 165.8 (C=O)ppm. Anal. Calcd for $C_{14}H_{11}F_2NO$: C, 68.01; H, 4.48; N, 5.67; found: C, 67.75; H, 4.59; N, 5.66.

4-Methyl-N-(4-methylsulfanyl-phenyl)-benzenesulfonamide (35)

Prepared as in method A from commercially available 4-methyl sulfonyl chloride (3.9 g, 0.019 mol) dissolved in acetone (50 mL) and 4-Methylsulfanyl-phenylamine (1. 39g, 0.01 mol) to yield title compound as white colored powder (2.1 g, 72%). mp: 110-111°C; (lit. mp = 111-111.5°C (Simonov and Chemagin,1953)). ν_{max} (KBr disc) 3318, 2910, 1589cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 2.2 (s, 3H), 2.35 (s, 3H), 7.21 (d, 2H, J = 8.6), 7.28 (d, 2H, J = 8), 7.69 (d, 2H, J = 8.6), 7.8 (d, 2H, J = 8), 9.81 (brs,1H, NH) ppm; ^{13}C NMR (75.48 MHz, DMSO): δ 15.97 (CH_3), 21.5 (CH_3), 121.5 (2xCH), 127.4(2xCH), 128.2 (2xCH), 129.4 (2xCH), 132.3(C), 132.7 (C), 137.2 (C), 142.1 (C)ppm. Anal. Calcd for $C_{14}H_{15}NO_2S_2$: C, 57.31; H, 5.15; N, 4.77; found: C, 56. 85; H, 5.20; N, 4.64.

N-(4-Chloro-2-methyl-phenyl)-4-methyl-benzamide (36)

Cl NH O

Prepared as in method A from commercially available 4-Methyl benzoyl chloride (3.1 g, 0.02 mol) dissolved in acetone (50 mL) and 4-Chloro-2-methylphenylamine (1.41g, 0.01mol) to yield title compound as white crystalline powder (2.3 g, 89%). mp: 145-146 °C (lit. Mp=146°C (Al-Nadaf *et al.*, 2010)). ν_{max} (KBr disc) 3335, 2919, 1620, 1594 cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 2.2 (s, 3H), 2.35 (s, 3H), 7.2-7.25 (m, 2H), 7.5 (d, 1H, J = 1.77), 7.29 (d, 2H, J = 8), 7.8 (d, 2H, J = 8), 9.81 (brs,1H, NH) ppm;^{13}C NMR (75.48 MHz, DMSO): δ 17.9 (CH_3), 21.5 (CH_3), 125.9 (CH), 126.3 (CH), 128.2 (2xCH), 129.5 (2xCH), 130.3 (C), 131.9 (C),132.3(CH), 132.8 (C), 138.4 (C), 142.3 (C), 165.8 (C=O)ppm. HRMS-ESI m/z $[M+H]^+$ calcd for $C_{15}H_{15}ClNO$: 260.08422,

found: 260.08403. Anal. Calcd for $C_{15}H_{14}ClNO$: C, 69.36; H, 5.43; N, 5.39; found: C, 69.58; H, 5.20; N, 5. 25.

N-3-Amino-phenyl-4-methyl-benzenesulfonamide (37, Aleksandrov, 1959**)**

O
S
N
H
O
NH_2

Prepared as in method A from commercially available 4-Methylbenzenesulfonyl chloride (3.8 g, 0.019 mol) dissolved in acetone (50 mL) and Benzene-1, 3-diamine (1.08 g, 0.01 mol) to yield title compound as white colored powder (2. 1g, 80%). mp: 230–233°C ;HRMS-ESI *m/z* $[M-H]^-$ calcd for $C_{13}H_{13}N_2O_2S$: 261.06977, found: 261.07032. Anal. Calcd for $C_{13}H_{14}N_2O_2S$: C, 59.52; H, 5.38; N, 10.68; found: C, 59. 31; H, 4.96; N, 10.08.

Benzoic acid 4-chloro-2-methyl-phenyl ester (38, Kenaga, 1949**)**

O
Cl
O

Prepared as in method A from commercially available benzoyl chloride (2.8 g, 0.019 mol) dissolved in acetone (50 mL) and 4-Chloro-2-methyl-phenol (1.42 g, 0.01mol) to yield title compound as white colored powder (1.8 g, 73%). mp: 88-89°C; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{14}H_{12}ClO_2$: 247.05258, found: 247.05233 . Anal. Calcd for $C_{14}H_{11}ClO_2$: C, 68.16; H, 4.46; found: C, 67.95; H, 3.99.

N-(4-Methoxy-3-methyl-phenyl)-4-nitro-benzamide (39)

Prepared as in method B from commercially available 4-nitrobenzoyl chloride (2.85 gm, 0.015 mol) dissolved in pyridine (50 mL) and 4-Methoxy-3-methylphenylamine (1.37 g, 0.01mol) to yield title compound as yellow colored powder (2.1gm, 80%). mp: 161–163°C; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{15}H_{15}N_2O_4$: 287.10318, found: 287.10273. ν_{max} (KBr disc) 3420, 1684, 1603cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 2.23 (s, 3H), 3.76 (s, 3H), 6.94 (m, 2H),7.51(s, 1H), 8.12 (d, 2H, J = 8.7), 8.3 (d, 2H, J = 8.7), 10.12 (brs,1H, NH) ppm;^{13}C NMR (75.48 MHz, DMSO): δ 20.8 (CH_3), 56.2 (CH_3), 111.9 (CH), 124.1 (2xCH), 126.1 (CH), 126.4(C), 127.1(CH), 129.5(C), 129.6 (2xCH), 140.8(C), 149.6 (C), 150.4 (C), 164.1 (C=O)ppm. Anal. Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79; found: C, 62.27; H, 4.52; N, 9.85.

4-Methyl-N-(4-methylsulfanyl-phenyl)-benzamide (40)

Prepared as in method A from commercially available 4- Methyl benzoyl chloride (3.1 g, 0.02 mol) dissolved in acetone (50 mL) and 4-Methylsulfanylphenylamine (1. 39g, 0.01 mol) to yield title compound as white colored powder (2.1 g, 82%). mp: 247.5–248.4°C; ν_{max} (KBr disc) 3339, 2914, 1650, 1590cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 2.35 (s, 3H), 2.46 (s, 2H), 7.2 (d, 2H, J = 8.6), 7.28 (d, 2H, J = 8.1), 7.69 (d, 2H, J = 8.6), 7.82 (d, 2H, J = 8.1), 10.12 (brs,1H, NH) ppm;^{13}C NMR (75.48 MHz, DMSO): δ

15.97 (CH_3), 21.5 (CH_3), 121.5 (2xCH), 127.4(2xCH), 128.2 (2xCH), 129.4 (2xCH), 132.3(C), 132.7 (C), 137.2 (C), 142.1 (C), 165.7 (C=O)ppm. HRMS-ESI *m/z* $[M-H]^-$ calcd for $C_{15}H_{14}NOS$: 256.07961, found:256.07956. Anal. Calcd for $C_{15}H_{15}NOS$: C, 70.01; H, 5.87; N, 5.44; found: C, 69.85; H, 5.52; N, 5.22.

N-(4-Chloro-2-methyl-phenyl)-benzenesulfonamide (41)

Prepared as in method A from commercially available sulfonyl chloride (3.5 g, 0.019 mol) dissolved in acetone (50 mL) and 4-chloro-2-methyl aniline (1.41 g, 0.01mol) to yield title compound as brown colored powder (1.89 gm, 67%). mp: 189°C; ν_{max} (KBr disc) 3235, 2919, 1608cm^{-1}; HRMS-ESI *m/z* $[M-H]^-$ calcd for $C_{13}H_{11}ClNO_2S$**:** 280.01990**,** found: 280.01945. ^1H NMR (300 MHz, DMSO): δ 1.89 (s, 3H), 6.74 (s, 1H), 7.11 (s, 2H), 7.36-7.82 (m, 5H), 9.79 (brs,1H, NH) ppm;^{13}C NMR (75.48 MHz, DMSO): δ 17.5 (CH_3), 126.2 (CH), 126.6 (CH), 127 (2xCH), 128.8 (C), 129.8 (2xCH), 131.5 (CH),132.7(CH), 136.7 (C), 138.5 (C), 140.6 (C)ppm. Anal. Calcd for $C_{13}H_{12}ClNO_2S$: C, 55.42; H, 4.29; N, 4.97; found: C, 54. 85; H, 4.02; N, 4.64.

N-Benzyl-3-nitro-benzenesulfonamide (42)

Prepared as in method A from commercially available 3-Nitro-benzenesulfonyl chloride (4.4 g, 0.02 mol) dissolved in acetone (50 mL) and Benzylamine (1.01 g, 0.01mol) to yield title compound as brown colored powder (1.76 gm, 60%). mp: 95-96°C; (lit. Mp=95-98°C (Kushner *et al*., 1954)). ^{1}H NMR (300 MHz, DMSO): δ 1.89 (s, 3H), 6.74 (s, 1H), 7.11 (s, 2H), 7.36-7.82 (m, 5H), 9.79 (brs,1H, NH) ppm;^{13}C NMR (75.48 MHz, DMSO): δ 42.9 (CH_2), 117.8 (CH), 122.4 (CH), 122.45 (CH), 123.5 (2xCH), 124.2 (2xCH), 124.7(C), 125.8 (CH), 127.9 (CH), 130.8 (C), 138 (C)ppm. Anal. Calcd for $C_{13}H_{12}N_2O_4S$: C, 53.42; H, 4.14; N, 9.58; found: C, 52. 97; H, 4.02; N, 9.04.

N-(4-Fluoro-phenyl)-3, 5-dinitro-benzamide (43)

Prepared as in method B from commercially available 3, 5-dinitrobenzoyl chloride (2.34 g, 0.015mol) dissolved in pyridine (50 mL) and 4-flouroaniline (1.11 g, 0.01 mol) to yield title compound as white colored powder (2.99 gm, 98%). mp:170-171°C; ν_{max} (KBr disc) 3289, 1642, 1587 cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 7.36 (m, 3H), 8.85 (d, 2H, J = 2), 8.96 (d, 2H, J = 2), 10 (brs,1H, NH) ppm;^{13}C NMR (75.48 MHz, DMSO): δ 122.5 (2xCH), 124.6 (CH), 129.4 (4xCH), 134.8(C), 137.2 (C), 148.8 (C), 148.8 (C), 149.6 (C), 164.5 (C=O)ppm. HRMS-ESI *m/z* [M−H]$^{-}$ calcd for $C_{13}H_7FN_3O_5$: 304.03697, found: 304.03694. Anal. Calcd for $C_{13}H_8FN_3O_5$: C, 51.16; H, 2.64; N, 13.77; found: C, 50.79; H, 2.60; N, 13.24.

N-(4-Methoxy-phenyl)-4-nitro-benzamide (44)

Prepared as in method B from commercially available 4-nitrobenzoyl chloride (2.34 g, 0.015 mol) dissolved in pyridine (50 mL) and 4-Methoxyphenylamine (1.23 g, 0.01 mol) to yield title compound as green colored powder (2.68 g , 99%). mp:197-198°C (lit. mp = 196-198°C (Serdons *et al.,* 2009)). ^{1}H NMR (300 MHz, DMSO): δ 3.72 (s, 3H), 6.89 (d, 2H, J = 9.03), 7.64 (d, 2H, J = 9.01), 8.13 (d, 2H, J = 8.8), 8.31 (d, 2H, J = 8.8), 10.41 (brs,1H, NH) ppm;^{13}C NMR (100 MHz, DMSO): δ 55.7 (CH_3), 114.3 (2xCH), 122.6 (2xCH), 124.01 (2xCH), 129.6 (2xCH), 132.2(C), 141.2 (C), 149.5 (C), 156.4 (C), 163.9 (C=O)ppm. HRMS-ESI *m/z* $[M-H]^-$ calcd for $C_{13}H_{11}N_2O_4$: 271.07188, found: 271.07143. Anal. Calcd for $C_{13}H_{12}N_2O_4$: C, 61.76; H, 4.44; N, 10.29; found: C, 61.27; H, 4.51; N, 10.25.

4-Methyl-N-phenyl-benzamide (45)

Prepared as in method A from commercially available 4- Methyl benzoyl chloride (6 g, 0.04 mol) dissolved in acetone (50 mL) and aniline (1.86 g, 0.02 mol) to yield title compound as off white colored powder (3.1 g, 74%). mp: 141-142°C (lit. mp = 141-142°C (Shi *et al.,* 2010)); HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{14}H_{12}NO$: 210.25118, found:210.25109. Anal. Calcd for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63; found: C, 78.85; H, 5.52; N, 6.22.

4-Nitro-benzoic acid 4-chloro-3-methyl-phenyl ester (46, Devani *et al.,* 1980**)**

Prepared as in method B from commercially available 4-nitrobenzoyl chloride (2.34 g, 0.015 mol) dissolved in pyridine (50 mL) and 4-Chloro-3-methylphenol (1.42 g, 0.01 mol) to yield title compound as white colored powder (2.87 g, 98%). mp:90-92°C. ^{1}H NMR (300 MHz, DMSO): δ 2.33 (s, 3H), 7.28 (m, 1H), 7.45 (d, 2H, J = 8.6), 7.55-7.8 (m, 2H), 8.1 (d, 2H, J = 8.5) ppm; ^{13}C NMR (75.48 MHz, DMSO): δ 20 (CH_3), 121.6 (CH), 124.9 (CH), 129.3 (C), 129.5 (2xCH), 130.2 (C), 130.3 (2xCH), 130.9 (C), 134.7 (CH), 137.5 (C), 149.8 (C), 164.9 (C=O)ppm. Anal. Calcd for $C_{14}H_{10}ClNO_4$: C, 57.65; H, 3.46; N, 4.80; found: C, 56.98; H, 3.35; N,4.32.

N-(3, 4-Dichloro-phenyl)-4-methyl-benzamide (47, Mitsutake *et al.*, 1986**)**

Prepared as in method B from commercially available 4-methyl benzoyl chloride (2.85g, 0.015mol) dissolved in pyridine (50 mL) and 3, 4-Dichloroaniline (1.62 g, 0.01 mol) to yield title compound as white powder (2.77 gm, 99%). mp:175-176°C ; ^{1}H NMR (300 MHz, DMSO): δ 2.3 (s, 3H), 7.03-7.07 (m, 1H), 7.23 (d, 1H, J = 2.45), 7.32 (d, 2H, J = 8.1), 7.4 (d, 1H, J = 8.8), 7.6 (d, 1H, J = 8.1), 10.61 (brs,1H, NH) ppm; ^{13}C NMR (75.48 MHz, DMSO): δ 21.5 (CH_3), 119.9 (CH), 121.2 (CH), 126.3 (C), 127.2 (2xCH), 130.4 (C), 131.7 (2xCH), 131.9 (CH), 136.5 (C), 138.6 (C), 144.3 (C), 164.9 (C=O)ppm. Anal. Calcd for $C_{14}H_{11}Cl_2NO$: C, 60.02; H, 3.96; N, 5.00; found: C, 59. 89; H, 3.53; N, 4.65.

N-(4-Methoxy-phenyl)-benzamide (48)

Prepared as in method B from commercially available benzoyl chloride (1.76gm, 0.015 mol) dissolved in pyridine (50 mL) and 3, 4-Dichloroaniline (1.62 g, 0.01mol) to yield title compound as white crystalline powder (2.1 g, 93%). mp:153-154°C (lit. mp = 153-154°C (Fors and Buchwald, 2010)). ^{1}H NMR (300 MHz, DMSO): δ 3.69 (s, 3H), 6.9 (d, 2H, J = 8.8), 7.2 (d, 2H, J = 8.8), 7.35-7.49 (m, 4H), 7.7 (d, 1H, J = 2.11), 10.41 (brs, 1H, NH) ppm;^{13}C NMR (75.48 MHz, DMSO): δ 55.8 (CH_3), 115.2 (2xCH), 128.9 (2xCH), 129.4 (2xCH), 129.9 (2xCH), 132.6 (CH), 132.9 (C), 135.4 (C), 158.9 (C), 173.5 (C=O)ppm. Anal. Calcd for $C_{14}H_{13}NO_2$: C, 73.99; H, 5.77; N, 6.16; found: C, 73.08; H, 5.59; N,5.94.

N-(2, 4-Difluoro-phenyl)-4-methyl-benzenesulfonamide (49*)

Prepared as in method A from commercially available 4-Methylbenzenesulfonyl chloride (3.8 g, 0.019 mol) dissolved in acetone (50 mL) and 2, 4-difluoroaniline (1.29 g, 0.01mol) to yield title compound as off-white colored powder (2.4 g, 85%). mp: 90°, HRMS-ESI m/z $[M+Na]^+$ calcd for $C_{13}H_{11}F_2NNaO_2S$: 306.03762, found: 306.03704. ν_{max} (KBr disc) 3240, 2921, 1603cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 2.32(s, 3H), 6.95-7.02 (m, 1H), 7.1-7.2 (m, 2H), 7.3 (d, 2H, J = 8.06), 7.5 (d, 2H, J = 8.27), 7.68 (brs,1H, NH)ppm; ^{13}C NMR (75.48 MHz, DMSO): δ 21.5 (CH_3), 104.5 (CH), 111.4

(CH), 128.3(2xCH), 128.9(C), 129.5 (2xCH), 130.4 (C), 142.4(CH), 158.2 (C), 161.7(C), 165.8(C)ppm. Anal. Calcd for $C_{13}H_{11}F_2NO_2S$: C, 55.12; H, 3.91; N, 4.94; found: C, 54. 93; H, 2.89; N, 4.61.

3.1. In-vitro Biological Assays

3.1.1. Materials and Instruments

- Wistar male rats, weighing between 140-180 gm and fed *ad libitum* with standard feed and water. They were cared for in the animal lab at faculty of Pharmacy at University of Jordan.
- Dimethylsulfoxide (DMSO) was purchased from Sigma-Aldrich Company.
- Diethyl Ether Anhydrous, ACS. Reagent, min. 99.0%, was purchased from TEDIA Company (USA).
- NaCl for molecular biology, Min. 98%, From Sigma, (USA).
- Hit compounds were synthesized in organic lab as part of thesis works.
- Bovine Serum Albumin (BSA).
- Collagenase was purchased from Sigma, (USA).
- Dithioerythritol (DTE), From Fluka, (Switzerland).
- Krebs Ringer Bicarbonate (KRB), Sigma, (USA).
- Tris base, from Promega Corporation Company, (USA).
- Protease inhibitors tablet (SIGMAFAST)
- *P*-Nitro Phenyl Butyrate (PNPB), from Sigma,(USA).
- Sucrose for molecular biology, 99.5%.,BioUltra, From Sigma, (USA).

- Acetonitrile, HPLC, Spectroscopy Grade, Min. 99.9%, was purchased from TEDIA Company (USA).
- NaH_2PO_4, Min. 98%, From Sigma, (USA).
- WTC binder static incubator (J.P. SELECTA, Spain).
- DAIGI shaking incubator (DAIGI SCIENCES CO., LTD., Korea).

3.1.2. Preparation of buffers

Many different buffers might be prepared before beginning extraction of HSL enzyme, a salt solution was the basic buffer for maintaining pH and osmotic balance and providing the cells water, nutrition and essential inorganic ions.

Three types of buffers might be prepared for extraction & biological assay:

I-Homogenization buffer:

For prepared 100 mL of this buffer you need 50 mM of Tris-HCL, 250 mM sucrose, and one tablet of crushed protease inhibitor. Firstly, weighted 0, 6057 gm of Tries-HCL and dissolved in 70 mL of double distill water, the pH of the buffer was adjusted to get pH around 7 (I got pH=6.97) by using 1N HCL or 1N NaOH. After that, one tablet of protease inhibitor (which contained 1mM of EDTA as chelating agent) was crushed to powder and dissolved with 8.5575 gm sucrose into 70 mL adjusted Tris-HCL buffer. Finally, the volume of buffer was completed to 100 mL of double distill water & store in cold condition about 2-8 ° c.

II. Krebs Ringer Bicarbonate Buffer (KRB).

For prepared 1L of this buffer you need one package of KRB, and 1.26g sodium bicarbonate. Firstly, measure out 900 mL of double distill water and worm it to get water with temperature 15-20 □ c, while gently stirring the water, add the KRB powdered , then adding 1.26 g of sodium bicarbonate, stirring until dissolved without heating. The pH of the buffer was adjusted to get pH 7.1-7.3 (I got pH=7.17) by using 1N HCL or 1N NaOH. Finally, the volume of buffer was completed to 1L of double distill water & sterilized immediately by filtration using filtration unit with membrane porosity of 0.22 micron(I used 0.45micron), store in cold condition about 2-8 ° c.

III. Krebs Ringer Bicarbonate - Bovine Serum Albumin Buffer (KRB- BSA).

It can prepare by weighted 4 gm of BSA and dissolved in KRB Buffer and complete the volume to 100mL of buffer.

3.1.3. Extraction of the HSL Enzyme

Isolated fat cells were extracted from rat epididymal adipose tissues as described earlier (Rodbell, 1964). Briefly, Wistar male rats (fed *ad libitum*) were immolated by cervical dislocation, and their epididymal fat pads were removed quickly and rinsed several times in normal saline. The tissue was weighed , cut and minced into small pieces. The resulting mass placed in a flask and treated with following solution: For each 1.0 g of tissue, 3 mL KRB (pH 7.4) supplemented with 4% BSA were added, followed by 10 mg collagenase.

The mixture was incubated and agitated in a metabolic shaker (Shaking Incubator, Daiki Scientific Corporation) over 2 hour at 37 °C. Subsequently, fat cells were liberated from the tissue fragments by gentle stirring with a rod three times.

The resulting suspension was centrifuged for 1 minute at 400 x *g* at 20 °C. Fat cells floated to the surface while stromal-vascular cells settled at the bottom. Stromal-vascular cells were removed by aspiration. Fat cells were decanted and washed by suspending them in 10 mL of warm (37 °C) KRB-BSA solution followed by centrifugation (for 1 minute at 400 x *g* at 20 °C) and removal of stromal-vascular cells by aspiration. This washing procedure was repeated three times.

HSL was extracted from epididymal fat cells (Morimoto *et al*., 1999). Briefly, 1 mL of suspended fat cells (in KRB-BSA solution) was further diluted by 2.5 mL KRB-BSA and incubated at 37 °C for 30 min. Subsequently, the suspension was centrifuged at 100 x *g* for 1 min to separate the infranatant from the fat cells.

For each 1.0 mL of suspended fat cells, a 1.125 mL-homogenization buffer (Each 100 mL prepared from 50 mM Tris-HCl, pH 7.0, 250 mM sucrose, and 1 crushed protease inhibitor tablet) was added and the mixture was manually agitated 20 times. The homogenate was centrifuged at 4,540 x *g* and 4 °C over 10 min. Subsequently, 250 μL diethyl ether was added to the homogenate and abruptly shaken and centrifuged at 1,200 x g over 5 min at 4 °C. The upper ether layer was aspirated. The subsequent supernatant was used as HSL extract. 0.5 mL-HSL extract aliquots were stored in Epindruff tubes at -80 °C for later use.

3.1.4 Preparation of Hit Compounds for *In Vitro* Assay

The tested compounds were provided as dry powders in variable quantities (20 or 30 gm). They were initially dissolved in DMSO to give stock solutions of 50 mM. They were subsequently diluted to the required concentrations with phosphate buffer (pH 7.25; 0.1M NaH_2PO_4; 0.9% NaCl and 1.0 mM Dithioerythritol) for subsequent enzymatic assay.

3.1.5 Quantification of HSL Activity in a Spectrophotometric Assay

The lipase activity of HSL was quantified by a colorimetric assay that measures the release of *p*-nitro phenol as previously described (Slee *et al*., 2003). However, *p*-Nitro phenyl butyrate (PNPB) was employed as HSL substrate at 10 µM in the enzymatic assays instead of 5 mM (Slee *et al*., 2003). 0.10 ml HSL extract was added to the reaction mixtures. The volume was completed to 1 ml using phosphate buffer before measuring the solution absorbance spectrophotometrically at λ of 400 nm at 3 time points: 1 min, 3 min and 6 min. The reactions were maintained at 37 °C.

3.1.6 HSL Inhibition by Hit Compounds

The inhibition of HSL lipase activity by the synthetic compounds was measured using the spectrophotometric assay described above. HSL was pre-incubated with 10 µM of each particular hit compound for 30 min at 37 °C before adding the substrate. The final concentration of DMSO did not exceed 1.0%. The percentage of residual activity of HSL was determined for each compound by comparing the lipase activity of HSL with and without the compound. The concentration required to give 50% inhibition (IC_{50}) was determined for the seven compounds having the best inhibitory activities. HSL was pre-incubated with different concentrations of the selected compounds (10, 31 and, 38 Table 1) and the percentages of residual activity of HSL data were used to evaluate the IC_{50} values.

4. Results and Discussion

4.1 Syntheses of Aryl and arylalkyl amides, sulfonamides, Sulfonates and Esters

One of the most important methods for synthesis of esters and amide is the coupling reaction of acyl chlorides, aroyl chlorides or sulfonyl chlorides with phenols, amines or their derivatives. The reaction is simple as sulfonyl or acyl chlorides are highly reactive acylating electrophiles that react very rapidly with phenols, amines or anilines. 4-Dimethylaminopyridine (DMAP) is commonly used as acylating catalyst and triethylamine (TEA) as acid scavanger.

The DMAP's role is the initial formation of an acylpyridinium ion, which then reacts with the attacking nucleophile (phenol or amine). DMAP has better nucleophilic properties compared to phenols or amines. On the other hand, the acyl-pyridinium ion is extremenly reactive electrophile that reacts rapidly with phenol and amines compared to acyl chlorides because of the positive charge of the pyridinium ion, which renders pyridine as excellent leaving group. The excellent nucleophilic properties of DMAP are because of the dimethylamino group that act as an electron donating substituent.

Accordingly, syntheses of title compounds (table 1) were based on nucleophilic attack of aniline, amine or alchohol moieties against reactive acyl or sulfonyl chloride derivatives. The reactions were performed in the presence of triethylamine as acid scavenger in the presence of acylation catalysts such pyridine and dimethylaminopyridine (DMAP). The reactions produced the title compounds as single products at yields ranging from 60-99%. The mechanisms of the reaction are shown in **schemes 3** and **4.**

Scheme 3: Synthetic route for synthesis of acyl amide or ester.

Scheme 4: Synthetic route for synthesis sulfonyl ester or amide.

4.2 **Strucutre-Activity Relationship of the prepared compounds.**

By looking at table 1, one can see some patterns connecting the structure of prepared compounds with their bioactivities. Clearly, all prepared compounds fit, to certain extent, pharmacophore hypotheses Hypo4/9 and Hypo8/7. Still, one observes siginifcant fluctuations in bioacitivity among prepared compounds. These flucutuation can be probably explained by factors other than their ability to map (fit) the binding models.

Apparently, although sulfonamides fitted the optimal pharmacophore models better that their amide counterparts, their bioactivities were slightly inferior than their amidic counterparts probably because of the higher hydrophilicity of sulfone linkers compared to amide linkers. Higher hydrophilicity should promote hydration instead of binding within the hydrophobic pocket of HSL. This trend can be seen in several examples in table 1: **11** (HSL Inhibition = 27% at 10 μM) *vs.* **13** (HSL Inhibition = 42 % at 10 μM), **35** (HSL Inhibition = 38% at 10 μM) *vs.* **40** (HSL Inhibition = 43% at 10 μM), **34** (HSL Inhibition = 36% at 10 μM) *vs.* **49** (HSL Inhibition = 16% at 10 μM), **9** (HSL Inhibition = 27% at 10 μM) *vs.* **23** (HSL Inhibition = 33% at 10 μM).

A similar trend can be noticed with the aromatic substitutents on both aromatic rings of the scaffold. Hydrophobic substituents on any or both rings seem to improve anti-HSL bioacitivity of the molecules. For example: **5** (HSL Inhibition = 38% at 10 μM) *vs.* **13** (HSL Inhibition = 42% at 10 μM) and **10** (HSL Inhibition = 52% at 10 μM); **13**(HSL Inhibition = 42% at 10 μM) *vs.* **22** (HSL Inhibition = 15% at 10 μM); **10** (HSL Inhibition = 52% at 10 μM) *vs.* **45** (HSL Inhibition = 22% at 10 μM); **22** (HSL Inhibition = 15% at 10 μM) *vs.* **26** (HSL Inhibition = 22% at 10 μM) and **23** (HSL Inhibition = 33% at 10 μM) and **31**(HSL Inhibition =

53% at 53 μM); **2** (HSL Inhibition = 31% at 10 μM) *vs.* **44** (HSL Inhibition = 24% at 10 μM) and **3** (HSL Inhibition = 0% at 10 μM) and **21** (HSL Inhibition = 0% at 0 μM).

Anti-HSL bioacitivity seem to tolerate single atom insertion between the aromatic rings and the central amide or linker. However, additional inserted atoms cause drastic reduction in anti-HSL bioactivity. For example: **1** (HSL Inhibition = 42% at 10 μM) *vs.* **20** (HSL Inhibition = 2.6% at 10 μM).

From the above discussion, one can conclude that the binding pocket of HSL is quite hydrophobic and is intolerant of hydrophilic substitutions.

Finally, founding three compounds 10, 31, and 38 as the most potent synthesized compounds, of anti-HSL IC_{50} = 10, 37 and 10 μM, respectively, and therefore can be used as leads for subsequent optimization.

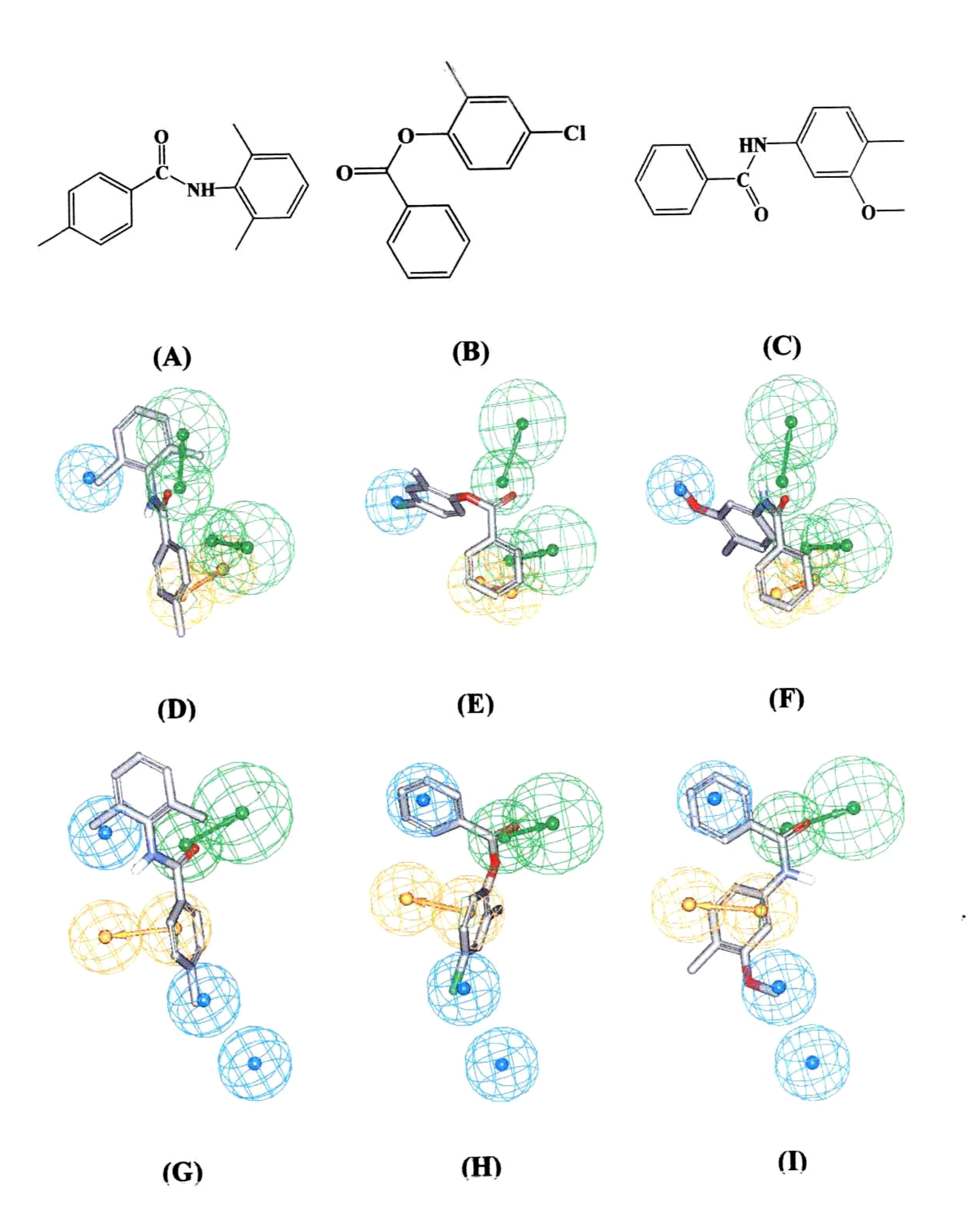


Figure 8: **(A), (B)** and **(C)** The most potent synthesized compounds, of anti-HSL IC50 = 10, 10 and 37 μM, respectively. (D), (E), and (F) show how the three compounds map Hypo8/7, respectively, while (G), (H) and (I) show how the three compounds map Hypo4/9, respectively.

Table 1: Prepared compounds, their fit values against binding models Hypo8/7 and Hypo4/9, and experimental anti-HSL bioactivities.

Cpd.	Structure	Yield	Fit Against		Experimental	
			Hypo8/7	Hypo4/9	% inhibition at 10 μM	IC50 (μM)
1		89	5.3 (HBA missed feature)	6.8 (Hbic missed feature)	42	ND[a]
2		97	2.6 (HBA missed feature)	1.33 (Hbic missed feature)	31	ND
3		76	2.4 (Hbic missed feature)	0	0	ND
4		82	4.8	0	0	ND
5		70	3.9 (HBA missed feature)	7 (Hbic missed feature)	38	ND
6		72	3.7 (HBA missed feature)	0	1	ND
7		81	3.7 (HBA missed feature)	6.9 (Hbic missed feature)	0	ND
8		90	5.3 (HBA missed feature)	0	0	ND
9		62	6.3	8.6 (Hbic missed feature)	27	ND
10		87	6.3	0	52	10 (R^2=0.9995)
11		87	4.9	5.7 (Hbic missed feature)	27	ND

Table 1: Prepared compounds, their fit values against binding models Hypo8/7 and Hypo4/9, and experimental anti-HSL bioactivities.

Cpd.	Structure	Yield	Fit Against		Experimental	
			Hypo8/7	Hypo4/9	% inhibition at 10 μM	IC50 (μM)
12		71	4.8 (Hbic missedfeature)	0	2	ND
13		93	4.8 (Hbic missed feature)	4.8 (Hbic missed feature)	42	ND
14		87	3.4 (HBA missed feature)	6.5 (Hbic missed feature)	39	ND
15		79	4.5 (HBA missed feature)	0	0	ND
16		85	4.4 (HBA missed feature)	0	15	ND
17		88	4.2 (HBA missed feature)	4.9 (Hbic missed feature)	20	ND
18		88	3.7 (HBA missed feature)	3.3 (Hbic missed feature)	21	ND
19		89	3.6	3.8	22	ND
20		89	5.2 (HBA missed feature)	5.5 (Hbic missed feature)	2.6	ND
21		77	2.6 (HBA missed feature)	6.5 (Hbic missed feature)	0	ND
22		91	2.9 (HBA missed feature)	5.5 (Hbic missed feature)	15	ND

Table 1: Prepared compounds, their fit values against binding models Hypo8/7 and Hypo4/9, and experimental anti-HSL bioactivities.

Cpd.	Structure	Yield	Fit Against		Experimental	
			Hypo8/7	Hypo4/9	% inhibition at 10 μM	IC50 (μM)
23		75	2.9 (HBA missed feature)	4.6 (Ring Arom missed feature)	33	ND
24		68	2.9 (HBA missed feature)	3.5 (Hbic missed feature)	25	ND
25		93	3.4 (HBA missed feature)	4.2 (Hbic missed feature)	33	ND
26		94	2.9 (HBA missed feature)	4.8 (Hbic missed feature)	22	ND
27		61	3.4 (HBA missed feature)	6.1 (Hbic missed feature)	0	ND
28		85	3.9 (HBA missed feature)	6.5 (Ring Arom missed feature)	13	ND
29		77	4.3 (HBA missed feature)	5.6 (Hbic missed feature)	0	ND
30		93	5.5 (HBA missed feature)	8 (Hbic missed feature)	0.2	ND
31		73	5 (HBA missed feature)	5.8 (Hbic missed feature)	53	37 (R^2=0.85)
32		83	6	8.6 (Hbic missed feature)	25	ND
33		71	6.3	8.3 (Hbic missed feature)	35	ND
34		77	3.4 (HBA missed feature)	5.7 (Hbic missed feature)	36	ND

Table 1: Prepared compounds, their fit values against binding models Hypo8/7 and Hypo4/9, and experimental anti-HSL bioactivities.

Cpd.	Structure	Yield	Fit Against		Experimental	
			Hypo8/7	Hypo4/9	% inhibition at 10 μM	IC50 (μM)
35		72	6.3	8.6 (Hbic missed feature)	38	ND
36		89	4.4 (HBA missed feature)	8.6 (Hbic missed feature)	32	ND
37		80	2.4	5.8 (Hbic missed feature)	34	ND
38		73	5.1 (HBA missed feature)	8.8 (Hbic missed feature)	52	10 (R^2=1)
39*		80	2.4 (Hbic missed feature)	0	45	ND
40*		82	3.4 (HBA missed feature)	6.6 (Hbic missed feature)	43	ND
41*		67	5.5 (HBA missed feature)	8.1 (Hbic missed feature)	28	ND
42		60	5.5	0	18	ND
43*		98	5.4 (Ring Arom missed feature)	0	15	ND
44		99	3.3 (HBA missed feature)	2.9 (Hbic missed feature)	24	ND
45		74	4.8 (HBA missed feature)	5.4 (Hbic missed feature)	22	ND

Table 1: Prepared compounds, their fit values against binding models Hypo8/7 and Hypo4/9, and experimental anti-HSL bioactivities.

Cpd.	Structure	Yield	Fit Against		Experimental	
			Hypo8/7	Hypo4/9	% inhibition at 10 μM	IC50 (μM)
46		98	5.4 (HBA missed feature)	6.9 (Hbic missed feature)	40	ND
47		99	4.5 (HBA missed feature)	5.1 (Hbic missed feature)	46	ND
48		93	3.3 (HBA missed feature)	5.3 (Ring Arom missed feature)	27	ND
49*		85	4.8	0	16	ND

*Those compounds are novel

[a] ND: Not determined

5. Conclusions

The current effort include preparation and biological testing of fortynine compounds of aryl amide, aryl ester and sulfonamide derivatives guided by previously published anti-HSL pharmacophore models and QSAR equation. The prepraration procedures included coupling reactions involving phenols, anilines and aryl alkyl amines with aroyl chlorides and arylsulfonyl chlorides. The reaction yields ranged from moderate to excellent. Three prepared compounds **10**, **31**, and **38** were found to possess micromolar anti-HSL properties, and therefore can be used as leads for subsequent optimization.

6. Future work

1- Exploring more lipophilic amids and sulfonamides as HSL inhibitors.

2- Selectivity studies against other enzymes; lipase.

3- Toxicological studies.

4- Human studies on the hits as potential hypoglycemic agents.

5- Screening and evaluation of other hits captured by the optimal pharmacophore.

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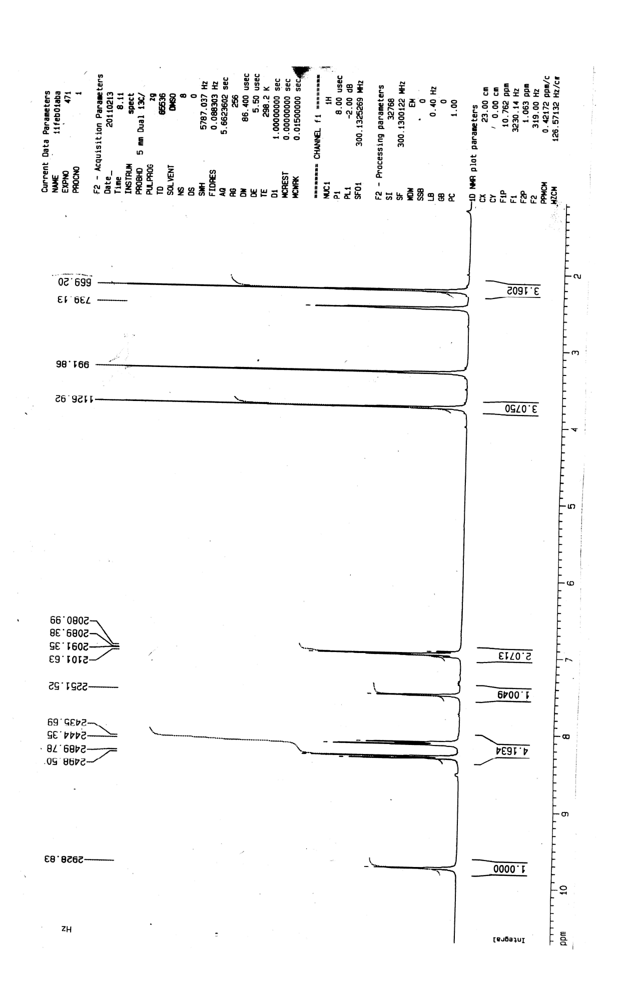
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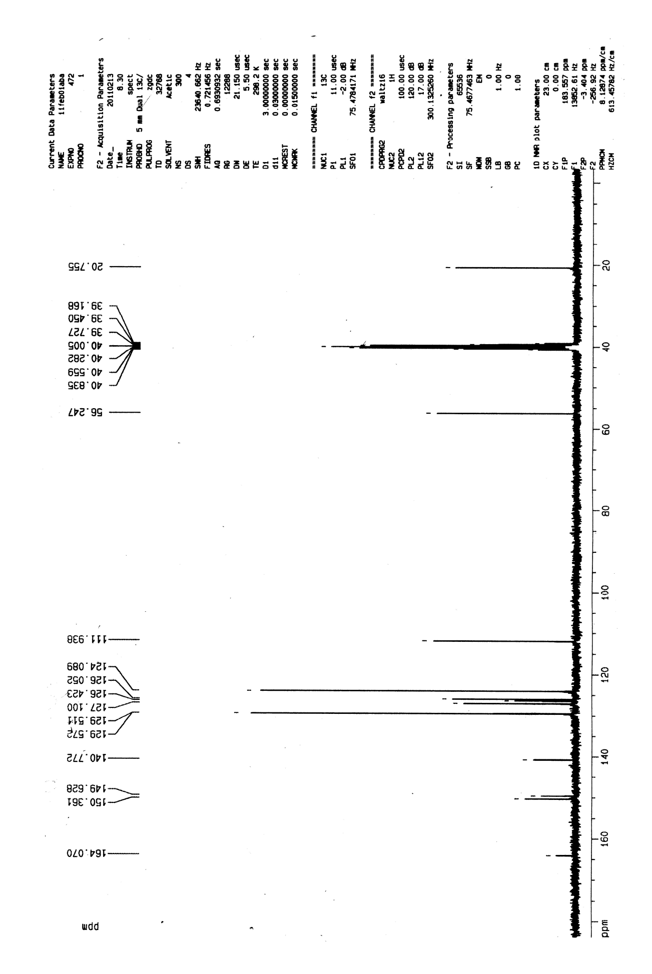
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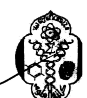
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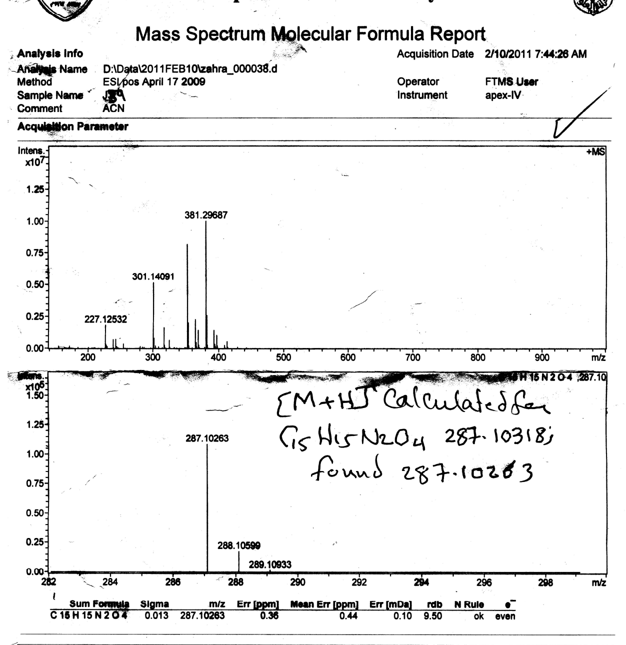


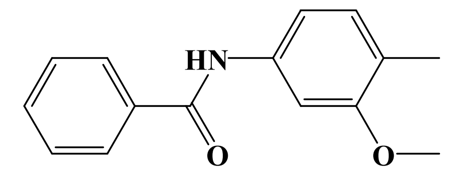




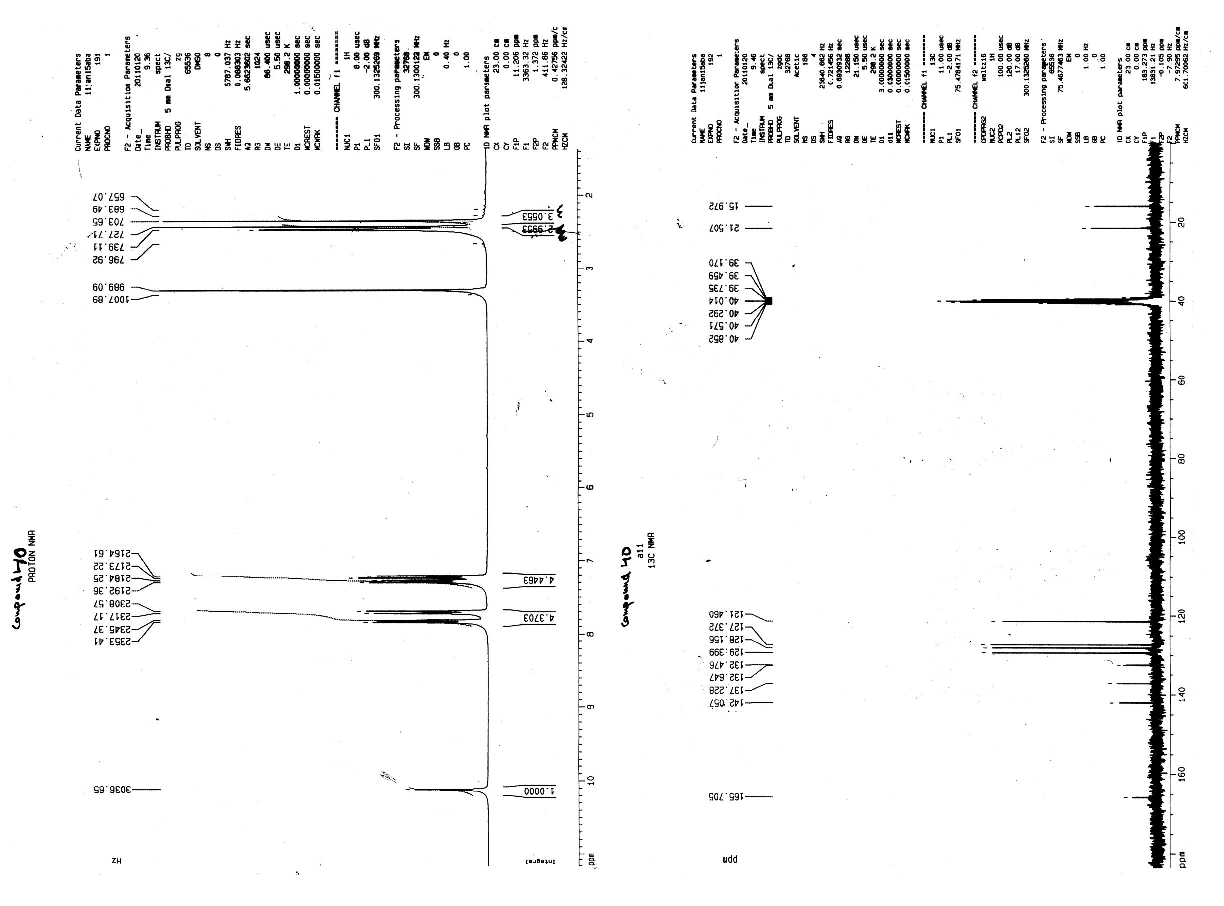
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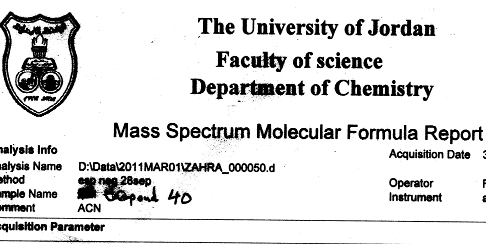






Compound **39**





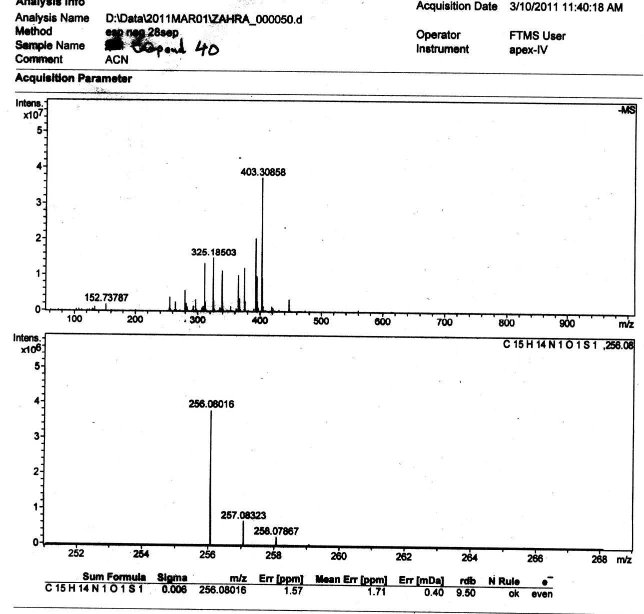


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Mass Spectrum Molecular Formula Report

Analysis Info			
Analysis Name	D:\Data\2011MAR01\ZAHRA_000050.d	Acquisition Date	3/10/2011 11:40:18 AM
Method	esp neg 28sep	Operator	FTMS User
Sample Name	Compound 40	Instrument	apex-IV
Comment	ACN		

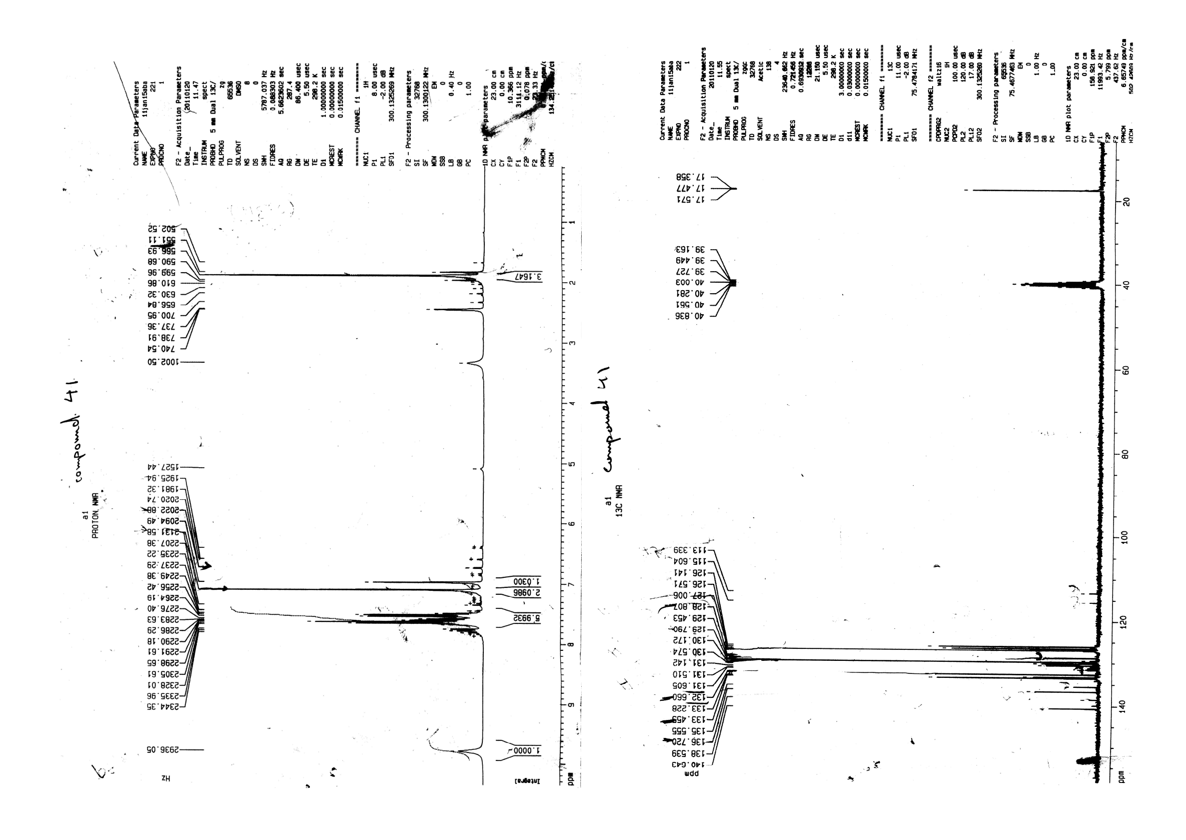
Acquisition Parameter

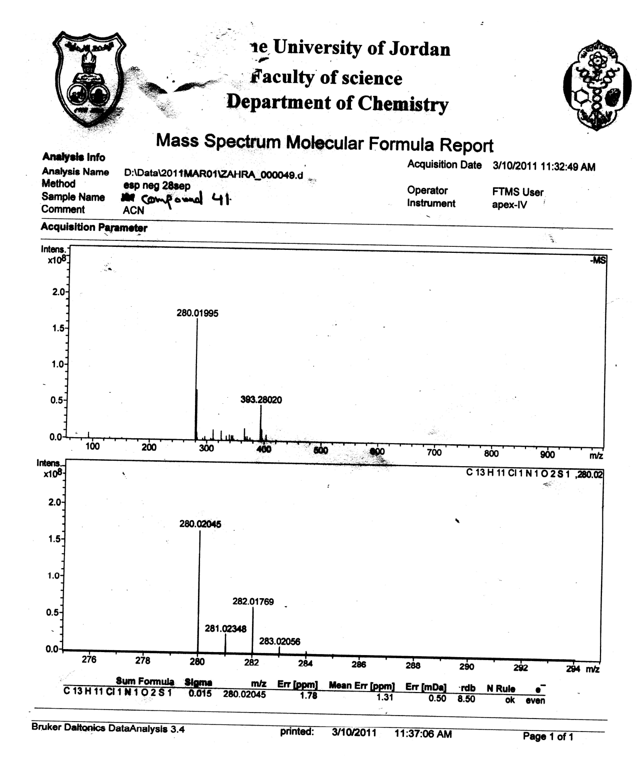


Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 15 H 14 N 1 O 1 S 1	0.006	256.08016	1.57	1.71	0.40	9.50	ok	even

Bruker Daltonics DataAnalysis 3.4 printed: 3/10/2011 11:43:48 AM Page 1 of 1

Compound **40**




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Mass Spectrum Molecular Formula Report
Analysis Info
Analysis Name D:\Data\2011MAR01\ZAHRA_000049.d
Method esp neg 28sep
Sample Name compound 41
Comment ACN
Acquisition Date 3/10/2011 11:32:49 AM
Operator FTMS User
Instrument apex-IV
Acquisition Parameter
280.01995
393.28020
C 13 H 11 Cl 1 N 1 O 2 S 1 ,280.02
280.02045
282.01769
281.02348
283.02056
Sum Formula Sigma m/z Err [ppm] Mean Err [ppm] Err [mDa] rdb N Rule e⁻
C 13 H 11 Cl 1 N 1 O 2 S 1 0.015 280.02045 1.78 1.31 0.50 8.50 ok even
Bruker Daltonics DataAnalysis 3.4 printed: 3/10/2011 11:37:06 AM Page 1 of 1

Compound **41**

تصنيع و تحسين مثبطات جديدة للهرمون الحساس لأنزيم الليباز كعامل منظم للسكر

إعداد
جمانة داوود عقلة الشوابكة

المشرف
الأستاذ الدكتور معتصم طه

المشرف المشارك
الدكتورغسان ابو شيخة

ملخص

بعد معرفة اهميته الهرمون الحساس لأنزيم الليبز ودوره المهم في السكري والسمنه, وإكتشاف الفراغ الفارماكوفوري المحوسب للأنزيم؛ مما جعلنا نهتم بتصنيع وتطوير مركبات جديدة سهلة التحضير كعوامل مثبطة للهرمون الحساس لإنزيم الليبز.
تم تحضير 49 مركب وفحصها الفراغ الفارماكوفوري المحوسب للأنزيم من ثم اجراء فحوصات حيوية عليها, وشملت المركبات المحضرة أميدات الاريل, وايسترات الاريل, و أميدات سلفونات الاريل, وايسترات سلفونات الاريل, وذلك بعد تطبيق النماذج الفارماكوفورية للهرمون. عملية التصنيع شملت تفاعلات بسيطة بين الفينولات ومشتقاتها, والأنلين ومشتقاته مع كلوريدات الأريل وسلفونات كلوريد الأريل. حيث تراوح المردود المئوي للتفاعلات 60-99%.

ومن الجدير بالذكر انه بعد عمل فحوصات حيوية على المركبات الناتجة وجد ثلاثة مركبات ذات فعالية عالية في تثبيط الهرمون الحساس لأنزيم الليبز. وتمت عملية ربط لمعرفة العلاقة بين تركيب المركبات المحضرة و شكل الموقع الرابط بالأنزيم.